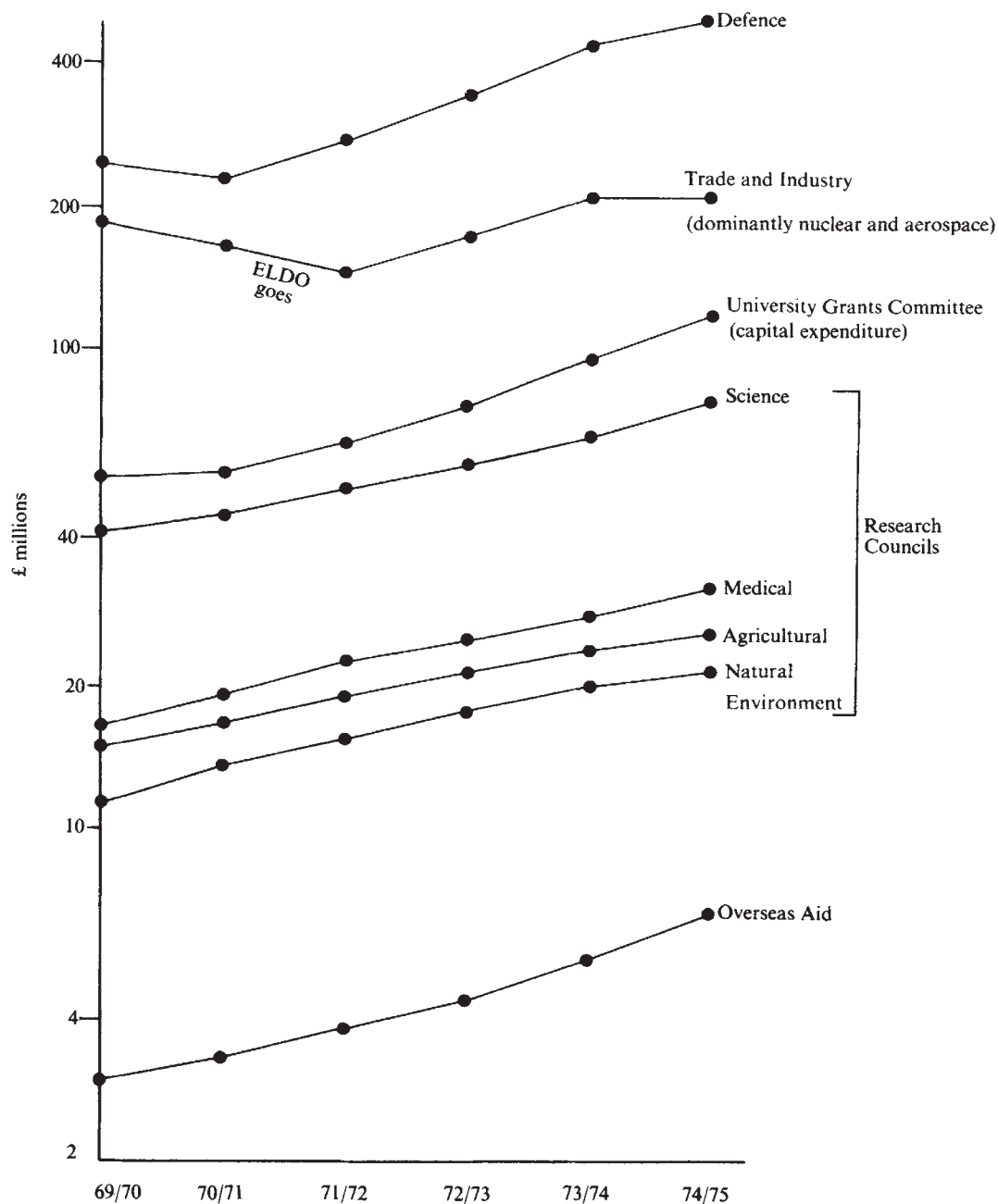




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How Britain spends its research and development money



Sources

Advisory Board for the Research Councils, First Report, Appendix V, Table 1, June 1974.

Memorandum by the Chief Secretary to the Treasury, 1974/75.

Adjustments have been made to 'de-Rothschild' research council expenditures for the past two years.



Chilean scientists remaining in the country are labouring under very difficult circumstances which are mainly economic. Funds for research are very limited, and it is very difficult for these people to carry out experiments using modern techniques.

From Carlos Eyzaguirre, Chairman of the Department of Physiology, University of Utah College of Medicine, Salt Lake City, and Visiting Professor, Department of Neurobiology, Catholic University of Chile, Santiago.

● I READ with interest a letter (*Nature*, March 29) signed by H. M. Gerschenfeld and others, regarding the situation of the academic community in Chile. It is intriguing and provoking since it shows a rather desperate situation: instances of police brutality, wholesale dismissals of faculty and students and a total disregard for academic freedoms and human rights on the part of the military government. The letter is inaccurate and the charges very serious. Thus, a reply is in order.

I started my academic career in Chile and went permanently to the United States about 17 years ago. Since then, I have returned to Chile many times for academic purposes and have continuously maintained close contacts with Chilean academics. At present I am a Visiting Professor of Neurobiology at the Catholic University and have been in the country since April. Thus, I feel better qualified to judge the present situation than the gentlemen signing the letter to *Nature*.

Dr Allende's Government started with high hopes for the underprivileged who are numerous in this country. Unfortunately, slowly and steadily things deteriorated to the point of chaos and anarchy. The universities were not immune to this decaying process: funds were drastically cut to the point where research came to a standstill. CONICYT (equivalent to the National Research Council), which actively supported research during the previous Frei administration, became a planning agency which developed a number of programmes more often than not politically motivated. The Academy of Sciences of the Instituto de Chile was left practically without funds. There was total lack of student discipline to the point where it was extremely difficult to conduct normal academic activities. In addition, there were frequent armed confrontations on the streets, incredible government corruption and, near the end, people had to queue for hours to get a few

essentials such as bread, soap, fuel, cigarettes. In the middle of this lovely picture the military struck with vigour on September 11, 1973. Interestingly enough they were shot at by supposedly defenceless civilians. In the melee many people were killed (including soldiers and policemen) and mopping up operations continued for a while. In context, the military operation in Chile was not a palace coup but a civil war which, fortunately, lasted only a few days. Casualties were many, but they could have been much more numerous if the military had been less decisive.

No one denies that there have been mistakes, cases of police harshness and unfair firing of people from their jobs. Some of these unpleasant practices have, however, been corrected; the country is again at work with the consequent increase in productivity, there is more discipline and normal activities have resumed. The people have cooperated, not necessarily because of fear, but the coup had the acceptance of the majority of the people. In a country like this, with a profound democratic tradition, nobody can govern without popular acquiescence. The democratic process has not been restored yet. Congress and the political parties are in recess and the press is still quite cautious. There is, however, hope for the future and the military have promised full democracy as soon as possible, whenever that may be. Many people believe that these are not vacuous statements since the military in Chile have been and are civic minded. Proof of this is that they have supervised all elections since 1938, when under President Pedro Aguirre (head of a Popular Front coalition) they got that authority. This practice continued under Dr Allende and there is consensus that elections in Chile have been generally fair.

Why did the military strike? They did so to restore the country to a more normal way of life, to eliminate the unconstitutional excesses of the previous government and the political, moral and economic chaos present in Chile at that time.

It was not an easy task, from a public relations point of view, since Dr Allende enjoyed great popularity in foreign countries. He was pictured as a democrat trying to reach socialism by peaceful and constitutional means,

a David fighting the United States Goliath and opening new ways of government to improve the lot of the poor; in short, Sir Galahad under a poncho. Most of his countrymen saw him in a different light: a scheming, corrupt and manoueuering politician trying to outflank the wishes of Congress and of the Courts and bribe the Armed Forces, and very incompetent in the business of running the country. Moreover, they resented the fact that Dr Castro and the Soviet Union had too much to say about national affairs. Some of these complaints were unfair since there was political and economic pressure from the United States and he had to turn somewhere for help. The fact remains, however, that he could not manage the affairs of the nation and Chile would have faced certain economic, moral and political ruin had he remained in power.

Soon after the military coup, the heads (rectors) of the different universities were fired by the government. They were replaced by retired senior armed forces officers. Also, an army general was made head of CONICYT. These appointments seem, on the surface, to go against traditional university procedures, at least in Chile where most university presidents have been academicians. In other countries such as the United States, university presidents vary from academic to scientific administrators (such as Dr Fletcher at the University of Utah and now head of NASA) or retired politicians or generals (Eisenhower at Columbia). Thus, the appointments of Admiral Swett as Rector of the Catholic University or General Ruiz at the University of Chile would not be too far from practices that are not unknown in the United States. The difference is that both Admiral Swett and General Ruiz were appointed very soon after the military takeover in Chile. Interestingly enough, Admiral Swett's appointment was confirmed by Cardinal Silva, head of the Catholic Church and Chancellor of the Catholic University.

The new university rectors faced several problems: (1) an unruly student body heavily loaded with Marxist activists. Some of them were not students in the traditional sense of being properly registered, attending classes, taking examinations and so on; (2) a

peculiar faculty, some members being almost exclusively interested in the propagation of Marxism and organising revolutionary and guerrilla activities. Some did not even have a BA degree. This situation became intolerable for some faculty members who left the country and sought employment abroad; (3) chaotic finances which prevented normal university activities and the payment of adequate personnel salaries. One wonders if any university in a civilised country would have tolerated that situation for long.

The rectors had to act and they did the following: (1) students at some universities had to re-register. Students who did not qualify as such in the traditional sense (taking a certain number of courses for credit, passing qualifying examinations and so on) were dismissed. This weeded out the purely political 'students', but is this so terribly unfair? At the Catholic University, however, all of 11,500 students remained and no one was required to re-register. (2) The faculty went through the same screening procedure. Those who did not qualify for lack of degrees or for being exclusively political activists were dismissed. This procedure would have been applied in any university under normal circumstances. Left-leaning faculty members who carried out their duties of teaching and research during Allende's government were not asked to resign. Many of them are still in Chile. Some have left because of their inability to come to terms with the present government. This is their prerogative and their exit from the country has not been impeded.

Wholesale executions of faculty or scientists because of political views have not occurred. Very few fell either immediately after or during the coup because, allegedly, they were caught either shooting at soldiers, carrying arms or actively participating in the organisation of revolutionary activities. For the past several months, however, there has been no indication that such a thing has occurred again. Some academicians, deeply involved in extremist activities were detained for investigation. Most of them have been freed and many have returned to jobs in the same or different universities. Others have either not returned to the country or have sought asylum. There



Rector Ruiz

are charges against some of these people and if they return they will face legal prosecution. But prosecution is one thing and persecution another. Some academicians have had trouble with the police because of denunciations by neighbours or colleagues. These people have been investigated and, if cleared, they have been employed again in the same or similar jobs.

The question is then, what constitutes an offence leading to academic dismissal that would prevent further academic employment? I am not privy to the deliberations of the rectors, but it is my opinion that such an offence would be a deliberate effort on the part of an individual to topple the present government by either propaganda or more violent means. In other countries these activities are not looked on with favour by the governments involved. Thus, it is asking rather too much of the present Chilean government to tolerate these activities, particularly since the military came to power only a short time ago and after a rather messy civil war. This deals directly with a statement in the letter to *Nature* regarding the Catholic University. The statement is inaccurate. Only 3.25% of faculty members were removed after the coup because they did not do teaching and research but engaged exclusively in different forms of political activity. To assume that the rest (96.75%) were all right-wing, God-fearing, church-going and thoroughly nice fellows would be naive in the extreme. In fact, many of them have views that would not please the present government. But Admiral Swett has been a careful, intelligent and conscientious administrator who

has strived very hard to maintain and improve the present academic structure. He has the confidence and respect of both faculty and students.

With regard to the Chilean scientists remaining in the country the following must be said. These people are labouring under very difficult circumstances which are mainly economic. They have very low salaries (which are in line with those of others), mainly because of the difficult economic conditions of the country caused by the previous government; recovery is still very incomplete. For the same reason, funds for research are very limited, and it is very difficult for these people to carry out experiments using modern techniques. Furthermore, subscriptions to journals in libraries are running far behind, also for lack of funds, which makes the situation doubly difficult. Nevertheless, Chilean scientists are trying very hard to maintain a research establishment that is in bad shape although it was flourishing four or five years ago. But there is hope. CONICYT is operating again, fulfilling the purpose for which it was originally created during the Frei administration. Also, the government has rescued the Academy of Sciences from financial disaster. More important is the fact that government authorities are listening and are sympathetic to the plight of the local scientists in terms of improving salaries and making available more funds for research and so on.

Inaccurate letters of the type published in *Nature*, although well meaning, do not help those still in Chile. They create a distorted image of the country that may prevent foreign help which is badly needed in keeping what is left after the debacle of the past three years. Scientists who remain in Chile are not fascists but people sincerely devoted in pursuing their academic careers and training future researchers. In fact, some have received tempting offers from abroad and have refused to leave the country. They are far more courageous than those who left to practice 'gauchisme de salon'. The latter and their supporters should be more accurate in their statements, as one would expect from scientists, since these shot-gun approaches only hinder the struggle of dedicated scientists working in a difficult environment. ●

international news

THE Central Policy Review Staff (CPRS), the Think Tank, surfaced this week to publish a report on Energy Conservation (HMSO, £1). It has already been estimated that Britain should eventually be able to save up to 10% of its forecast energy consumption and the report details how this may be most usefully achieved. With its eyes firmly on the possible, it eschews long term once-and-for-all solutions such as nuclear fusion and the efficient use of solar energy, and concentrates on the small scale short to medium term answers. The electric car is a firm favourite; and better insulation to reduce heat loss, smaller cars, greater use of diesel rather than petrol engines and a reliance on the self-regulating effect of more expensive fuel all figure as feasible ways of conserving our energy over the next 25 years.

The most important conclusion for British research is a negative one, that Britain cannot conceivably follow up fully all the possible answers. The report does, however, pick out some fields in which practical research should be stepped up. For the rest, we should concentrate on monitoring the work of other countries, mainly though not exclusively through the Energy Technology Support Unit at Harwell. One of the favourite topics for further research in Britain is the electric car. For this to be feasible it is assumed that the electricity to recharge the batteries will by then be generated mainly by nuclear power.

Three ways of powering the electric vehicle are considered: hybrid systems

Get cracking and count the kilowatts

comprising a battery and a small internal combustion engine, fuel cells, and electric batteries. Of these, electric batteries are considered to be the most attractive. Further work is certainly justified, the report thinks, on the sodium-sulphur battery, to establish its technical viability and costing.

The only strong contender to replace fossil fuels in electricity generation is nuclear fission. Solar power and nuclear fusion, along with windmills, tidal power and magnetohydrodynamics are dismissed as too long term. But wave power is given the go ahead and the technical and economic appraisal now in progress at the National Physical Laboratory is welcomed.

One major recommendation, which has been increasingly advocated by the architectural profession, is an improvement of insulation standards in new domestic housing, and encouragement of owners of existing housing to bring their insulation up to scratch. The insulation in many British homes would not be acceptable to a modern pig farmer for his pig houses, says the CPRS, and there is therefore great scope for improvement in this direction. The Health and Safety at Work

Bill, now going through Parliament, will in fact enable the insulation standards in new housing to be raised. A massive programme to educate the public is envisaged, and this will probably concentrate on the house owner's pocket.

No policy can hope to reduce energy consumption drastically in the very near future. Any measures that the government do put in hand will be unlikely to show results until after the 1980s, especially as the emphasis of the report is on voluntary and self-regulating measures. No energy police for instance. The report has no revolutionary suggestions, but points out that relatively small individual savings, as a result of, for instance, better insulation, more efficient electricity generating equipment and the use of smaller motor cars, should eventually be able to save the target 10% of energy consumption, provided that they are acted on now as a matter of urgency.

The message from the top is, therefore, that people must be made conscious of the full cost of energy and the opportunities for conservation. This should encourage them to save their own money and is preferred to the strong-arm approach. One idea for industry is that companies should be encouraged to start 'energy auditing' and even publish the results in their annual reports. The psychology of economic self interest and the dislike of public disapproval are obviously considered to be more powerful in the long run than a corps of Energy Inspectors or Temperature Police.



BRITISH Secretary of State for Energy, Eric Varley, has appointed Dr Walter Marshall (left) as Chief Scientist in the Department of Energy. Initial reactions of relief that a key post has been filled after months in caretaker hands have been tempered somewhat by the details of the new man's brief, which says specifically that Dr Marshall shall not hand out any advice on overall nuclear policy or on UK Atomic Energy Authority (UKAEA) matters unless he is specifically requested to do so by the Department of Energy with the prior agreement of the UKAEA.

Since Dr Marshall has been a member of the UKAEA for two years, and

Director of the Atomic Research Establishment at Harwell since 1968, it seems on the face of things that the government is missing the point of the operation by directing his attention to energy issues excluding those which he is uniquely equipped by his experience to judge.

However, the choice of reactor for the next stage of Britain's nuclear programme has probably been made already, and whatever the department may say about Dr. Marshall's brief avoiding 'conflicts of interest', it's highly unlikely that he wasn't among the people consulted by Mr Varley, however informally, before the choice was made.

WHEN President Nixon abolished the Office of Science and Technology last year, thereby ending 15 years of scientific presence in the White House, his action was greeted with a predictable flood of complaints from the scientific community and rumblings of discontent have continued ever since. Now the National Academy of Sciences, the most prestigious scientific body in the United States, has taken to print to deplore the banishment of scientists from the corridors of power and to urge that they be restored through the establishment of a Council for Science and Technology in the White House.

The idea is neither new nor particularly startling, but the fact that it has come from the academy has accorded it considerable attention, particularly on Capitol Hill. The proposal is, essentially, for a three-member council to be established to help in dividing the budgetary pie for science and technology, to provide independent analyses of military research and development programmes and to have a strong input into foreign and domestic science policymaking.

When Nixon scrapped the Office of Science and Technology (OST) by executive fiat in January last year, he also designated Dr H. Guyford Stever, Director of the National Science Foundation, as science adviser—a position previously occupied by the Director of OST. Stever, who is head of one of the smaller scientific agencies in the federal landscape, was, however, given no authority to advise on military research and development—which accounts for more than half of the federal science budget—because “the Department of Defense has strong capabilities for assessing weapons needs”. And he was also told to report to the President through Dr George Shultz, who was then Nixon’s adviser on economic affairs.

Although Stever has generally been credited with doing a commendable job, given the limit to his powers, the fact that the science advisory apparatus was shifted from the White House to a relatively obscure part of the federal bureaucracy rankled considerably among members of the scientific establishment. And it did not help matters much when Nixon, seeking advice on energy research and development, turned not to Stever but to Dr Dixy Lee Ray, chairman of the Atomic Energy Commission. Since that was perhaps the most important exercise in science policymaking last year, the fact that Stever was seemingly overlooked when the task was assigned suggested that Nixon was not paying much attention to the science policy machinery that he had established.

Central to the complaints that have

Scientists want a foot in the White House

by Colin Norman, Washington

been raised about the new arrangements for science policy is that although Stever may be an able man, and although he has established an office to provide staffwork on policy questions, the fact that he is not in the White House puts him in a weak position to orchestrate the federal government’s scientific activities, which are scattered over numerous different agencies and departments. The White House is the place where interagency disputes are settled, where coordination of programmes takes place and, most important, where final decisions on the Administration’s budget are taken. Thus, the argument goes, for scientific advice to be effective it must come from a White House office.

That, in short, is the reasoning behind the academy’s proposal for a Council for Science and Technology. The proposal, which was released during Congressional hearings last week recommends the following features for the Council for Science and Technology:

- It would consist of at least three people, appointed by the President; it would have a small staff, and it would be able to call upon consultants and panels of outside scientists for advice.
- The council would establish strong links with the three chief policymaking organisations in the White House—the Domestic Council, which provides coordination and policy guidance for a range of domestic programmes, the National Security Council, which does the same for defence matters, and the Office of Management and Budget, which holds the purse strings for all government departments and agencies.

The academy suggests that the chairman of the Council for Science and Technology should sit on the Domestic Council, since that body must deal with “a substantial number (of policies and problems) which involve components of science and technology”. Arrangements with the National Security Council would be more informal, but participation by the proposed Council for Science and Technology in defence policymaking would at least rectify one of the major deficiencies in the present science policy system—lack of independent scrutiny of Defense Department programmes. As for the Office of Management and Budget, that body establishes priorities among federal programmes and agencies through the annual budget. Thus, the academy argues, it is essential that it be provided

with strong input from the scientific community in its deliberations over the federal government’s \$20,000 million science budget.

- Finally, the academy recommends that the proposed council should deliver an annual report on major developments in science and technology. In fact, OST did attempt to produce such a report during its last year of life, but the effort was eventually dropped partly because it engendered considerable opposition from some sectors of the government.

Nowhere in its report does the academy say outright that the present system is not working properly—indeed, at one point, the report says that “we view with admiration the efforts of the Director of the National Science Foundation”—but implicit in its argument is that the arrangement just cannot cope with many of the problems with which it has to deal, simply because it is one step removed from the centre of power. But a fundamental question is whether any science advisory apparatus can be made to work in an Administration which has not so far shown much enthusiasm for science advice. The OST arrangement, for example, even though it was at the centre of power, lost considerable influence during the later stages of its life, partly because PSAC made some public recommendations which were diametrically opposed to Administration policy—a prime example being a report opposed to development of the SST which was made public when the Administration was fighting Congress to get approval for the SST programme.

Nevertheless the Academy’s proposals met with a warm reception on Capitol Hill, for last week Senators Frank Moss and Warren Magnuson proposed a bill which would create a Council of Advisers on Science and Technology in the White House. Hearings will be held on the measure on July 11 by the Senate Committee on Aeronautical and Space Sciences and the Senate Committee (which Moss and Magnuson, respectively, chair) and there is a good chance that the Senate will endorse the proposal by the end of the year. But the House of Representatives is unlikely to move that quickly. The Committee on Science and Astronautics is now in the middle of a protracted study of the national science policy machinery, and although the Academy’s recommendations went down well with the committee, it is unlikely that it will be ready to suggest changes in the machinery in time for the House to act this year. But there is every chance that Congress will act next year to reestablish a White House science policy office, in which case no President would be able to get rid of it without congressional approval.

Cooperation in coal research

by John Wilson

BRITAIN and the United States are to share the results of almost all their research into the mining and use of coal. This was decided formally on June 26, when an agreement was signed simultaneously in London by Sir Derek Ezra, Chairman of the National Coal Board (NCB) and in Washington by the Honorable Rogers C. B. Morton, United States Secretary of the Interior. Over the live telephone link from Washington Mr Morton said that it was "not only feasible but imperative" that the two countries exchange information now. For his part, Sir Derek described the agreement as "about the most comprehensive list that could have been laid down". He emphasised, however, that the agreement should not be seen as being exclusive, and mentioned commitments to other countries.

The full text has not yet been released but fifteen major areas of research are specifically named. These are the gasification, hydrogenation and solvent extraction of the coal; long wall mining techniques (needed in deep mines); automatic cutting machine control; coal preparation techniques; automatic measurement of coal characteristics; effluent control; minestone disposal; noise and dust control; safety research in the workings (degasification, subsidence studies, roof supports, and prevention of spontaneous combustion); air pollution control (treatment of flue gases); open cast mining techniques (including restoration of the site); advanced power systems and fluid bed combustion.

How cooperation on many of these topics should best be established is not yet finalised but further discussions are expected to take place at an international meeting on coal research which is to be held in London in October.

The agreement runs initially for three years and is extended automatically for periods of two years unless one of the parties withdraws. Each side must call for the information it wants and there are provisions in the agreement for the safeguard of patents and the exchange of personnel.

With Sir Derek Ezra in London was Dr William Gouse, Director of the United States Office of Coal Research and Development and Acting Director of the Office of Coal Research. Saying that coal "was something that the United States had discovered last fall" he described its importance to the United States as an energy source of its own from which clean fuel could be prepared. But he said that there was a shortage of people with the appropriate research background to exploit

these resources—a weakness in mining technology being the weak link.

As part of President Nixon's drive to make the United States independent of external energy sources by 1980, the Department of the Interior plans to spend about \$3,500 million on mining and coal utilisation research over the next five years. And it hopes that this sum will almost be matched by private industry.

Britain will spend a lot less. In plans which Sir Derek Ezra says have been "well received" by the Secretary of State for Energy, Mr Eric Varley, the NCB is calling for \$100 million (£40 million) to be made available over the same period.

But this disparity in expenditure should not be taken as a measure of each country's contribution to the research agreement. Sir Derek Ezra says that "this agreement will be very much a two-way process. We are not just latching on to a great American effort, we have much to give in return". The NCB's knowledge of the basic processes in coal utilisation methods is sound, he continues, and the United States has the resources to set up the necessary pilot schemes.

The United States is particularly interested in the fluid bed combustion of coal—a technique in which the bed of coal and ash inside a furnace is given the properties of a liquid by the upward passage of air. It hopes to use this method to generate electricity and is spending \$30 million next year on the building of a pilot plant. Under the agreement just signed, the results from this and other similar pilot projects will be available to Britain.

The United States has only recently discovered that it will have to dig deep for its coal. This is not only to obtain sufficient quantities of high grade coal but also to avoid the wrath of the environment lobby which is violently opposed to strip mining.

Noting that Britain has by far the biggest mining industry in Western Europe, Sir Derek suggests that the NCB can assist the United States with the long-wall mining technique that it must now use—perhaps by the export of specialised mining equipment. The NCB also has considerable experience of the health problems posed by noise and dust in deep mines (indeed over the past four years Britain has allocated £4.5 million to medical and engineering research connected with dust control and pneumoconiosis) so there are many areas of both mining and coal utilisation research where Britain's expertise will benefit "Project Independence". But perhaps the most important aspect of the new agreement is that it will allow ideas generated on either side of the Atlantic to be shared between both parties.

Copper exploration proposed for Exmoor

by Roger Woodham

BRITISH Kynoch Metals Ltd. has applied for planning permission to explore for copper on the borders of the Exmoor National Park in Devon. The company, jointly owned by Imperial Metal Industries and British Insulated Callender's Cables, is thinking in terms of scout drilling of ten holes less than 350 feet deep to start with, but would ultimately seek permission to re-establish a mining operation in the area if things turn out well.

The first thing to be said is that open-cast mining is out of the question because of the nature of the mineralisation. The metal ores in the region are in lensoid bodies that are relatively narrow and lie *en echelon*. There is not the large extent of relatively poor grade ore (less than 1%) that makes open-cast mining attractive.

British Kynoch is hoping to prospect on "a few square miles" of the Stucley estate at Heasley Mill in the North Molton area of Devon. This part of the estate includes several old workings, in particular the Bamfylde Mine which was used intermittently between the early eighteenth century and 1884. Its output was never very great by today's standards, however—between 1860 and 1881 it produced 5,000 tons of copper ore, the grade of which may have been 17% or so locally but was probably more like 5-7% overall. The mine goes down 900 feet and the main vein is some 2,000 feet long.

Mr P. F. A. Loffler, Managing Director of British Kynoch, said last week that geochemical and geophysical surveys had provided "some encouragement". These methods are, however, no substitute for drilling and examining a core because copper can find its way into the soil from artificial sources and the detection by geophysical methods of conducting material at depth may indicate no more than the presence of iron ore or graphite, hardly a prize for any mining concern.

Mr Loffler said that as the area is well wooded and made up of valleys and combs, the operations could easily be screened. His company has declared that the "visual impact of equipment, plant and offices would be minimal" in the event that mining was started again and that no local smelting is contemplated.

Local reaction is hard to gauge at present because nobody has had time to investigate fully the implications of the planning application. There is, however, general relief that open-cast operations are not being considered for the site.

THE unofficial Moscow seminar on "Collective Phenomena and the applications of physics to other fields of science", scheduled for July 1-5, 1974 now must pass into history as "the seminar that never was". With the arrest on Friday June 28 of Sinologist Vitali Rubin, it would seem that all leading members of the organising committee were in custody at the time of going to press. According to a cousin of Professor Mark Azbel, the arrested organisers are held outside Moscow, in detention centres which have previously been used for the temporary removal of dissidents from circulation. The centres mentioned are at Volokolansk and Serpukhov—the second showing a certain irony on the part of the authorities. Those intending participants not in custody are under virtual house arrest with police cars parked outside.

Seminar that never was

On hearing of the arrests, Professor Edward Stern, one of the three international secretaries of the seminar, began an intensive campaign in Washington to effect the scientists' release. A delegation from the intending participants met Senators Jackson, Javits and Ribicoff, who undertook to send a telegram to Dr Kissinger, asking for their release. They also met Senator Hartke of Indiana who claims to be on good terms with Brezhnev, and who promises to intercede personally on their behalf. Acting Secretary of State Sisco also showed considerable interest in the plight of the scientists, and undertook to bring it to Dr Kissinger's

attention. Eleven Nobel Laureates endorsed the principle, which has been conveyed to Mr Nixon in Moscow, that the right of scientists to emigrate without harassment should be written into any bilateral agreement between the United States and USSR.

Meanwhile, it has been learned that Mrs Nina Voronel was informed on Saturday June 29 that the group will be released after the scheduled seminar dates have safely passed. What their subsequent fate will be remains uncertain. One of them, Corresponding-Academician Venyamin Levich, has, however, now been granted permission to emigrate "at the end of 1975", while his sons Venyamin and Aleksandr can leave at the end of this year. If this is an omen of events to come, the ill-fated Seminar will not have been convened in vain.

It is ten years since the June 1964 elections to the Soviet Academy of Sciences which marked the beginning of the end of Lysenkoism and the re-introduction of Mendelian genetics in the Soviet Union. Although the Russians are usually eager to mark any significant anniversary, this particular one is not liable to receive the usual acclamation of celebratory meetings and publications. Nevertheless, a recent decree of the Central Committee of the Communist Party of the USSR and the Council of Ministers of the USSR does, in oblique fashion, form a kind of epitaph to the Lysenko period, by indicating the harm done to the development of Soviet science by almost a quarter of a century of opting out of world trends in research in genetics and molecular biology.

The Decree, published on May 21, 1974, deals with "the question of measures to accelerate the development of molecular biology and molecular genetics and to use their achievements in the national economy". It begins with the face-saving observation that "in recent years, on the basis of the wide use in biology of the achievements of chemistry, physics and mathematics, it has become possible to investigate the molecular mechanisms of the most important processes determining the existence and development of living matter". (This would logically imply that the earlier decision, in 1948, to stop all genetic research and, on the orders of the MGB—now the KGB—to destroy all *Drosophila* held in laboratories by drowning them in boiling water, was quite 'correct'; the necessary achievements in other fields had not yet been reached.)

Now, however, "fundamental discoveries in this branch of the natural sciences" have been made (it is not said by whom), which "have great

Epitaph to Lysenkoism

from Vera Rich

theoretical and practical significance for the development of agriculture, medicine and a number of branches of industry". Nevertheless, it is found that "the general level and scale of research on molecular biology and molecular genetics in our country is still insufficient. There are only a few highly qualified specialists making ready in this field. There are serious deficiencies in the organisation of the production of special scientific instruments and high class apparatus, the necessary range of chemical reagents, materials and biological preparations."

Accordingly, since "the Central Committee of the Communist Party of the Soviet Union and the Council of Ministers of the USSR consider that the achievement in the shortest possible time of the foremost level of development of molecular biology, molecular genetics and other branches of natural science immediately connected with the study of the physico-chemical principles of life phenomena" constitutes "one of the most important problems of Soviet science at the present time", the appropriate steps are to be taken. The Academy of Sciences, the State Committee for Science and Technology, the State Planning Committee and the various ministries and departments are charged with "ensuring the necessary rate of development of these sciences and a wide use of their achievements in agriculture, medicine and industry", with "strengthening the basic trends of fundamental research", and also with drawing up a concrete programme of research and design for 1974-80.

This last clause is extremely signi-

ficant—the new policy becomes effective immediately, without waiting, as would normally be expected, for the beginning of the next five-year plan in 1976. Since the logistics of implementing such mid-plan changes of policy in the framework of the rigid quinquennial budgeting of financial and manpower resources are considerable, it seems clear that what is involved is not only a change of planning policy, which could have waited another 18 months, but something close to panic measures.

The practical details contained in the Decree are sparse, as always. We learn, however, that a special Interdepartmental Scientific and Technical Council has been formed to coordinate research in this field, that means have been assured for the training of specialists, scientists and instructors, that new scientific research establishments and training colleges are to be opened and existing ones expanded, and also that "research bases" are to be constructed. The production of the required instruments, reagents and other necessities is to be "considerably expanded".

The Decree ends with the conventional expression of the confidence of the Central Committee and Council of Ministers that all persons and organisations concerned will carry out their appointed tasks in this new expansion of Soviet science in the fields of molecular biology and molecular genetics. In this atmosphere of all shoulders to the wheel, it seems unfortunate, for the Soviet authorities, that they can no longer call on the help and assistance of the chronicler of the Lysenkoist "pseudoscience", who did so much towards its overthrow—Zhores A. Medvedev, who, a year ago, they saw fit to deprive of his passport.

In India the explosion is seen very differently

from Narender K. Sehgal, Jullundur

The news on May 18 of India's nuclear explosion sent a wave of elation through the country. People were simply thrilled, despite the difficult economic situation. Even Indians abroad could not remain unaffected by the event. The President of the Indian Students' Association at the University of Illinois in Chicago wrote in a letter to a New Delhi daily newspaper: "... If anything, the reaction of the average American has been one of incredulity as to how such a country could pull off something like this. ... The Indian community by and large welcomed the blast as a veritable success for Indian scientists and engineers". Back home, to show their appreciation, the Federation of Jullundur Engineering Associations passed a resolution announcing a token cash award of Rs2100 for Shri H.N. Sethna, Chairman of the Atomic Energy Commission (AEC), for "successful explosion of a nuclear device".

The Indian scientific community, like other sections of the population, hailed the underground nuclear experiment as an important development in the country's atomic energy programme, signalling attainment of a certain degree of sophistication in nuclear technology. Dr S. M. Sircar, Director of the Bose Institute in Calcutta, said that the Atomic Energy Commission had done a good job. Dr D. Bose, Director of the Indian Association for Cultivation of Science, said it was a good step forward in making use of atomic energy for purposes other than war. Dr S. P. Ghosh, Head of the Department of Nuclear Chemistry at Patna University in Bihar, said India's success in the field of nuclear technology represented peaceful research activities for geological exploration. He added that in the peaceful use of nuclear energy there was also a possibility of constructing atomic weapons and this explosion shows that "Indian technologists are capable of doing so."

Following the explosion, atomic energy in general and the country's programme in this area in particular became hot topics for discussions, talks and seminars. Dr H. S. Hans, Head of the Department of Physics at the Panjab University in Chandigarh, hailed the nuclear experiment in a radio talk from Jullundur and explained various aspects of India's atomic energy programme. Speaking on "Nuclear Explosion" at the India International Centre in New Delhi Dr B. K. Nayar, Executive Secretary of the Indian National Science Academy,



said the success achieved by India in the nuclear explosion must be a matter of both envy and dismay to many. He felt that so far as application of nuclear energy for peaceful purposes in India was concerned, a better experiment might not be easily envisaged. He stressed that a crater-forming explosion could be utilised successfully in creating channels, dams and storage for underground gases and radioactive materials released by nuclear tests.

Reactions to the explosion from abroad have been on expected lines. The Chairman of the French Atomic Energy Commission has congratulated his Indian counterpart. According to scientists, India had merely staged a neat and skilful demonstration of what she had been believed capable of for some time. Even so, there has been criticism from some foreign quarters, mostly in the form of unofficial press comments.

Although there is undoubtedly an understanding here about stands taken by non-nuclear countries, protestations from those possessing stockpiles of nuclear weapons have failed to impress anyone. Doubts have been cast on India's peaceful intentions in regard to the explosion, pointing out rather irrelevantly that even Russia and the United States have had little success in using nuclear explosions for peaceful purposes. Maybe India will be able to show the way.

By and large scientists here seem satisfied with the countrywide favourable response evoked by the nuclear experiment. There is concern, however, especially among those connected in any way with the atomic energy programme, at the patently false impression (created by press and other comments from abroad) that the entire Indian effort in the field of atomic energy had been directed to-

Shot in the arm

ward detonating a nuclear device. Nothing could be further from the truth. India's atomic energy programme comprises activities ranging over a wide variety of fields: radio isotopes, medicine, food and agriculture, electronics, lasers, minerals, metallurgy and so on—and of course power generation, which has been one of AEC's major concerns.

In a television interview on May 31, of the AEC, the Chairman, Shri Sethna, pointed out that the commission was spending seven times more on nuclear research in agriculture, medicine and cancer studies than what was spent on conducting the test which, according to him, cost the AEC Rs 30 lakhs (lakh = 100,000).

To Indian science the explosion has come as a timely shot in the arm. This modest achievement (and perhaps others that may follow) could provide just the fillip needed to bring Indian science and technology into their own.

Indian scientists feel that India's mastery over nuclear explosion technology for peaceful purposes is bound to result in important advantages: India's nuclear neighbour will feel appropriately restrained and chances of confrontation between the two will diminish; Indian views on disarmament, particularly with respect to nuclear weapons, will carry a lot more weight now; the idea and concept behind the nuclear Non-Proliferation Treaty will have to undergo adequate changes to make it more just for the non-nuclear-weapon countries; and India may now be in a far better position to press for initiation of discussion on dismantling of all existing nuclear weapons and on a worldwide ban on all types of nuclear activity for war purposes.

"The choice before mankind is a very simple one: nuclear disarmament or oblivion. In the absence of disarmament, more and more nuclear weapon powers will emerge. A limited nuclear war will eventually break out which may well escalate to a strategic nuclear exchange between the superpowers and extinguish our civilisation. The nuclear paradox is Man's total inability to cope with the obvious, even when it is a matter of life and death for the human race." With these words Dr Frank Barnaby, Director of the Stockholm International Peace Research Institute (SIPRI), justified the pessimistic outlook of the Institute's *Yearbook 1974* recently published in Stockholm. Like its four predecessors, this yearbook is about armaments and disarmament. After surveying the state of the armed world and the developments affecting it during 1973, the institute restates its commitment to general and complete disarmament as the only possibility for survival.

SIPRI readers have by now learned to expect the authoritative and sobering picture which emerges from this yearbook as from the institute's many other publications. It comes as no surprise to read that "... by the end of 1973 there was still little evidence that the degree of disorder in international affairs was decreasing". The bright spots of the year fall into place as acts of political necessity which have done nothing to stop the proliferation of conventional weapons or the continuing pressures towards technological refinement of both conventional and nuclear arms.

In 1973 the world spent \$207,000 million on arms, of which \$20,000 million was for military research and development. Since 1968 world military expenditure has been constant at about this level; therefore the latest figure represents a slightly lower proportion of world GNP now than it did then.

SIPRI pulls it all together

The unchanged figure however, disguises a disturbing trend: "The share of world military expenditure absorbed by the United States, the Soviet Union, France and the United Kingdom, taken together, has declined from 82% in 1955 to 70% in 1973. This does not reflect a reduction in the military capability of these four countries but rather indicates the magnitude of the increase in militarisation elsewhere." India's recent nuclear explosion illustrates this only too well.

The peculiar value of the SIPRI year books is that they relate all the happenings of a year, putting the events hailed as political milestones in perspective beside continuing world trends. Amidst the triumphant conclusion of the Israeli-Syrian disengagement agreement and the optimistic forecasts of its effect on future oil deliveries, for example, it is easy to overlook what is happening in other parts of the Middle East. The Persian Gulf is a critically important area for the shipment of oil. In recent years, the countries bordering the Gulf have, according to the yearbook, dramatically increased their military expenditures. Iran and Saudi Arabia have led the way. Over the decade 1963-1973 the average annual rate of increase of military expenditure in these countries was 23%. During 1973, Iran had outstanding orders for about 800 Chieftain main battle tanks, 250 Scorpion light tanks, more than 200 F-4E/F-5E fighter aircraft and nearly 500 helicopters, including about 200 Sea Cobra gunships armed with the latest US anti-tank missiles. The prospect of the Gulf countries being armed to the teeth and about to wield such influence

over oil shipments may well make us rethink the current relief over developments in another part of the Middle East.

Current nuclear events make the yearbook's discussion of nuclear policies particularly interesting. The statement on January 10 by the United States Secretary of Defense that the United States would in future pursue a counterforce strategy is interpreted by SIPRI as a justification for continuing the development of weapons for whose future deployment the doctrine of 'mutual assured destruction' provided no rationale. Of particular concern is the fact that enormous resources are being devoted to antisubmarine warfare technology, which is already sufficiently advanced to allow the destruction of a portion of the enemy's strategic nuclear submarine force. On the tactical level, it has been suggested that accurately delivered low yield nuclear weapons should replace the higher yield nuclear weapons now deployed, particularly in Europe. SIPRI objects to this proposal because "it is of paramount importance that an absolute 'firebreak' should be maintained between nuclear and conventional war".

It is surprising that SIPRI emphasises its basic commitment to effective disarmament as the only sure way of lessening threats all over the world, in spite of the facts that the yearbook is aimed largely at governments and that the institute is well aware that 'disarmament' is a dirty word in many government circles. The official intolerance of disarmament detracts from the practical value of the Yearbook's solution, for history shows that political will is essential for negotiated progress in any direction. In going against the stream, SIPRI is not merely being politically naive. It is attesting to the fact that it sees no middle way between disarmament and annihilation.

THE US House of Representatives voted last week to kill the Large Space Telescope (LST)—one of the most important astronomy programmes being planned for the 1980s—by deleting funding for the project from NASA's budget. If the Senate follows suit, NASA will have to revamp the project and come up with a cheaper option, but according to congressional sources, it is likely that the Senate Appropriations Committee will restore at least some of the money to keep LST alive.

NASA had asked for some \$6.2 million for the 1974-75 fiscal year for planning and design of the LST, before moving into the development phase. But the Appropriations Committee recommended that the money should

LST in danger

be denied this year and that "A less expensive and less ambitious project be considered as an alternative." The recommendation was approved by the House itself last week when it voted on NASA's budget.

A 120-inch optical telescope, the LST would be about 100 times more powerful than the largest ground-based instruments now in operation. It would be launched in the early 1980s—probably in 1981—by the space shuttle and it would be periodically serviced and upgraded during its 15-year lifetime. It has been enthusiastically endorsed by the Space Science Board of the

National Academy of Sciences but the House Appropriations Committee was evidently concerned at the large estimated costs of the project—between \$200 and 300 million.

Now that it has been approved by the House, NASA's budget must be considered by the Senate. The Senate Appropriations Committee is likely to report out a bill later this month and if the full Senate does eventually restore funding for the LST, the matter would have to be resolved by a House-Senate conference committee. Congressional sources predicted that the funds would be approved by the Senate and agreed to by the conference committee, but the outcome at this stage is far from certain.

news and views

Single mutation with two effects in tRNA

SINCE suppression was first discovered by the isolation of mutations that allowed some transfer RNAs to respond to nonsense codons instead of to their usual nucleotide triplets, the effects which mutational alteration may have upon tRNA have proved to be one of the most useful probes for investigating its function. The classical suppression of ochre (UAA) and amber (UAG) nonsense codons proved to be due to substitutions in the anticodons of tRNAs for glutamine, leucine or tyrosine, all possessing codons related to the nonsense triplets by a single base change. Missense suppression can result from a similar phenomenon, when a tRNA suffers a change in its anticodon which causes it to recognise a codon that usually represents some other amino acid. Another form of suppression involves frameshift mutations, in which a mutant tRNA restores the correct reading frame of the message, apparently acting at the codons GGG or CCC; whereas tRNA for glycine, for example, usually recognises GGG, a mutant seems able to respond to GGGG.

All these mutations are dominant; the presence of mutant tRNA achieves suppression. But what happens in the haploid bacterium at codons to which the mutant tRNAs would formerly have responded? When dominant suppressors can be isolated, presumably more than one tRNA must recognise their wild type codons, so that the capacity to translate these triplets remains even after mutation of one of the tRNAs. This explains why only some of the amino acids possessing codons related by single changes to the nonsense triplets can generate suppressors—presumably the others are recognised by only a single, essential tRNA whose mutation is lethal.

But the objection to mutation in essential tRNAs is overcome if the cell is made diploid for these genes; as Soll and Berg (*Proc. natn. Acad. Sci. U.S.A.*, **63**, 392; 1969; *Nature*, **223**, 1340; 1969) showed, in this case further suppressors can be isolated and they are recessive-lethals—if the bacterium is returned to a haploid condition, the suppressor tRNA is lethal, because there is no tRNA able to respond to its former codons. One of the two recessive-lethal suppressors that they isolated, *su7⁺*, has the surprising property of inserting glutamine at amber codons, surprising because dominant glutamine suppressors have previously been isolated and recessive-lethals were expected to implicate new amino acids in suppression. The unusual properties of the glutamine lethal-recessive suppressor are the subject of studies reported in the *Journal of Molecular Biology* by Soll (**86**, 233; 1974) and Yaniv, Folk, Berg and Soll (*ibid.*, 245).

Until very recently, all suppression—nonsense, missense or frameshift—mediated by tRNA seemed to be due to changes in the sequence of the anticodon that change the coding response of the transfer molecule. But Hirsh (*J. molec. Biol.*, **58**, 439; 1971) found that a UGA suppressor results from a mutation at position 24 of tryptophan tRNA, not located in the anticodon but allowing the molecule to respond to UGA as well as to its usual codon, UGG. And mutations affecting not the codons recognised by tRNA but instead the amino acid with which it is charged were recently isolated by Smith and Celis (*Nature new Biol.*, **243**,

66; 1973) and Celis *et al.* (*Nature new Biol.*, **244**, 261; 1973; see also *Nature*, **249**, 690; 1974). By examining the ability of cells possessing the *su3⁺* gene, which inserts tyrosine at amber codons, to suppress nonsense mutations at which tyrosine is not acceptable, they isolated mutants of *su3⁺* which continue to recognise the UAG amber codon, but insert glutamine instead of tyrosine. All five mutations constituted single base substitutions in the amino acid acceptor arm of the tRNA.

That the insertion of glutamine by the *su7⁺* suppressors results not from mutation of a glutamine tRNA but instead from a change in the charging specificity of another tRNA is the conclusion supported by the studies of Soll and Yaniv *et al.* Soll reports that the *su7* locus in *Escherichia coli* is identical to a locus in *Salmonella typhimurium* at which Miller and Roth (*J. molec. Biol.*, **59**, 63; 1971) previously isolated recessive-lethal suppressors of UAG and UGA codons that seem to be allelic. By obtaining a $\phi 80$ phage carrying the *su7⁻* gene, Soll showed that the *E. coli* locus also can suffer single step mutations to yield *su7⁺*_{UAG} or *su7⁺*_{UGA} suppressors. That both suppressors result from mutation of a single tRNA gene carried on the phage is suggested by the subsequent conversions possible from the

Is Jupiter lord of the Solar System?

THE idea that Jupiter and the Sun might be considered more sensibly as a binary system than in the respective roles of humble planet and dominating star is not particularly new. But on page 35 of this issue of *Nature*, Drobyshevski takes the idea an intriguing step further; according to his calculations, the dominant partner in the early stage of the development of the binary was Jupiter, and not the Sun.

Jupiter is certainly more like a small starlike body which has insufficient mass to trigger nuclear burning than like the other planets of the Solar System. According to Drobyshevski, one can be more specific and identify Jupiter with the core of the protosun. The process by which the original primary in the evolving binary system has become very much the secondary will be familiar to students of stellar evolution. At one time, it was something of a puzzle that the lower mass components in many binary star systems are found to be more highly evolved than their more massive companions, since a more massive star should evolve more rapidly. But this has now been explained in terms of mass exchange between the components of binary systems.

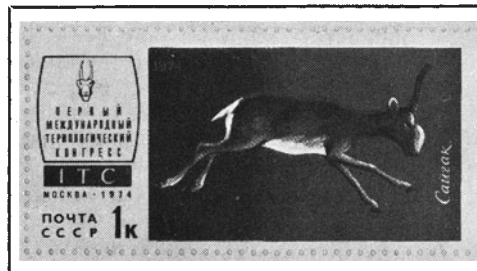
That is essentially how Drobyshevski explains the evolution of the early Solar System, with details of the process also offering a reasonable explanation of the formation of the other planets. And as a final bonus, the slow rotation of the Sun also emerges from the calculations when the angular momentum of the material streaming between the original binary companions is considered.

JOHN GRIBBIN

$su7^{+}_{UAG}$ and $su7^{+}_{UGA}$ alleles. The $su7^{+}_{UAG}$ tRNA can be mutated to yield an ochre suppressor recognising UAA and UAG. The $su7^{+}_{UGA}$ tRNA can be mutated to yield a UAG suppressor, although this results in loss of UGA suppression. These conversions can all be explained by single base mutations if the $su7^{-}$ tRNA represents the single species in the cell coding for tryptophan that responds to UGG. This can generate a UGA suppressor by mutation at position 24 in the dihydrouridine loop; and a change in the anticodon would generate a recessive-lethal UAG suppressor. Except for the initial generation of $su7^{+}_{UGA}$ from $su7^{-}$ tRNA^{Trp}_{UGG}, all the conversions observed can be achieved by single changes in the anticodon.

Sequencing studies confirmed that the amber and ochre $su7^{+}$ suppressors are derived by single base changes in the anticodon of the tryptophan tRNA. Yaniv *et al.* found that a change from CCA in the wild type anticodon to CUA in the mutant $su7^{+}_{UAG}$ is the sole difference between the transfer RNAs. The $su7^{+}_{UAA/G}$ suppressor generated by mutation of $su7^{+}_{UAG}$ represents a further single base substitution, having an anticodon of UUA. But although derived from tryptophan tRNA, the $su7^{+}_{UAG}$ and $su7^{+}_{UAA/G}$ suppressors both insert glutamine at the nonsense triplets to which they respond. This means that the mutation from C to U at the middle position of the anticodon must have two effects: it changes the coding response so that the tRNA recognises UAG instead of UGG; and it alters the charging specificity so that the transfer molecule is recognised by glutamine-tRNA synthetase instead of tryptophan-tRNA synthetase. The second C to U change, generating the ochre $su7$ suppressor, must change the coding specificity to allow recognition of UAA as well as UAG, but leaves unimpaired the new ability of the molecule to be charged with glutamine instead of tryptophan.

Comparing the sequences of the two glutamine tRNAs (which recognise CAG and CAA and differ in seven nucleotides) with the $su3^{+}$ tyrosine suppressor shows few similarities. All five of these mutations are located in the amino acid acceptor stem of the molecule. Four disrupt one of the first two base pairs, and may therefore be effective because they induce a conformation change; the other replaces an adenine not involved in base pairing with a guanine, and presumably this base must be recognised directly, or must influence the conformation of the molecule by an interaction with some other region in the tertiary structure. Each individual mutation may establish some similarity with glutamine tRNA and Inokuchi, Celis and Smith (*J. molec. Biol.*, **85**, 187; 1974) recently showed by constructing a double mutant that two together may enhance charging with



The saiga antelope, chosen as the emblem of the First International Theriological Congress and depicted on one of a special set of postage stamps issued to mark the event.

glutamine compared with the single mutants.

In view of the implication of the amino acid acceptor stem as a site for recognition by gln-tRNA synthetase, it may perhaps be significant that the only similarities between glutamine tRNA and $su7$ tryptophan tRNA lie in the base pairs of this region and of the anticodon stem, although of course recent research has pointed to the conclusion that base pairs as such, not their individual bases, may be important in recognition of tRNA. It is not at all obvious why a single change in the $su7^{-}$ tRNA anticodon allows the molecule to be recognised by gln-tRNA synthetase. Perhaps the isolation of further mutants, and determination of the relative extents to which each substitution allows false recognition of tRNA, may allow some definition of how synthetases recognise transfer molecules; but at present this remains unclear.

BENJAMIN LEWIN

Saiga finds safety in numbers

from D. Michael Stoddart

THE story of the saiga (*Saiga tatarica*) is one which could easily have had an unhappy ending. Intense piratical hunting and severe 'djuts' (glazed snow coverings of great persistence) brought the world's total of this antelope during the first quarter of this century to just a few hundred. In the 1930s the game authorities in the Soviet Union totally prohibited hunting and took steps to reduce the herds of nomadic cattle that overgrazed the saiga's range. Nature lent a kindly hand by offering a few winters of relative mildness without serious djuts. By 1954 there were 1 million head of saiga and in 1973 more than 2 million in the Soviet Union. Cropping started in 1955 and during the next 17 years nearly 3 million head were harvested and these produced 58,000 tons of meat. Current studies in Kazakhstan and other areas in which saiga overwinter are being made to find ways of lessening the impact of serious djuts.

An important feature of the ecology of the saiga is the high reproductive

rate. Old females regularly give birth to twins, sometimes triplets, and young females breed first at 7 months. The rate of embryo resorption is low but noticeably higher after winters with severe djuts when the crust of glazed snow diminishes available food and the animals enter spring in an emaciated state. In concluding his remarks to the First International Theriological Congress held in Moscow from June 6–12, A. A. Sludsky drew attention to the importance of this natural resource to the more isolated regions of the Soviet Union where rail and road transportation links are poor.

A less rosy outlook was presented by I. I. Barabash-Nikiforov and L. V. Shaposhnikov for the Russian desman (*Desmana moschata*), a large, aquatic shrew-like mammal. Like its Pyrenean cousin, the desman is a Tertiary relict. Everywhere it is becoming much less abundant. Today it is to be found only on parts of the River Ob' in West Siberia. Various reasons are thought to have brought the desman to the brink of extinction. Foremost among these is the use by fishermen of creels and trap nets in which many desmans drown. The draining programme of flood plains and the building of levées, the destruction by cattle of riverside vegetation and the interspecific competition with muskrats for bank space are other influential factors. It seems as if the economic and human factors will prevail and even spread to the few safe desman pockets where the creatures can find safe harbourage. Unless special reserves, like the Kljazma Reserve which was abandoned in 1951, are created and recreated soon, the desman will fast become another species only to be found in zoos.

An experimental farm for moose (*Alces alces*) at Kostroma, some 200 miles north-east of Moscow, is leading the way to a new line of domestic meat-bearing species. Bottle feeding of moose calves, group rearing and feeding are giving promising results. Moose cows breed in captivity when they are 16 months old. Their calves grow at a rate of about 300 g per day. On the farm they keep up this rate throughout the year, though wild calves do not usually increase in weight at all during the winter. E.M. Dzhurovich and A. P. Mikhailov are confident that a significant new branch of livestock hus-

bandry is just around the corner.

The congress was not concerned solely with mammals from the Soviet Union, nor just with ecology and production biology. About 1,000 zoologists from many countries of the world took part in a range of seminars and symposia which covered topics such as zoogeography, evolution and taxonomy, endocrinology and gestation, growth physiology, ethology, orientation and signalling, and the problems to be overcome in conserving bears and marine mammals. The USSR Academy of Science (see *Nature*, **249**, 502; 1974) organised the meeting in its 250th anniversary year. The congress has significantly fostered a closer spirit of interaction and cooperation between Soviet and Western zoologists than has hitherto been the case.

Antibody manipulation by malaria parasite

from F. E. G. Cox
Parasitology Correspondent

WITH more than one thousand million potential malaria victims in the world it is not surprising that considerable attention has been paid to possible methods of vaccination against this disease. All the attempts so far, however, have been relatively unsuccessful. All have involved killing or attenuating malaria parasites and using large amounts of antigen. Even then, the results have been variable and protection has been limited. Many workers believe that vaccination against malaria is not possible because of the existence of antigenic variation and cell mediated responses as yet unrecognised. In fact, much of the basic information necessary for the development of a vaccine is already available and it may well be that investigations of antigenic variation and cell mediated responses are irrelevant to immunity to clinical malaria.

Immunity to malaria can be explained in terms of an antibody directed against the merozoites while they are outside the red blood cells. The evidence for this comes from three sources. First, *in vitro* experiments carried out by Cohen and his colleagues at Guy's Hospital Medical School have demonstrated that parasites within their red cells take up ^3H -leucine in the presence of immune sera as well as they do in normal sera (*Trans. R. Soc. trop. Med. Hyg.*, **65**, 125; 1971) but that in immune sera the invasion of fresh blood cells is inhibited. They have also shown that merozoites bind to suitable cells (*Nature*, **244**, 40; 1973) and the prevention of this binding by immune

sera could form the basis of immunity. Second, Jerusalem, Weiss and Poels, in a paper that has not received the attention it deserves (*J. Immunol.*, **107**, 260; 1971), showed that fluorescein-labelled antiserum injected intravenously into mice bound only to merozoites and not to intracellular parasites. Third, nobody has observed any damage or signs of changes in parasites within their cells during the immune response.

If it is only the merozoites that are important in immunity, why is it that the antibodies associated with antigenic variation agglutinate infected cells? Part of the answer to this question comes from the work of Brown (*Nature*, **242**, 49; 1973) and Brown and Hills (*Trans. R. Soc. trop. Med. Hyg.*, **68**, 139; 1974). They have found that in *Plasmodium knowlesi* infections in monkeys, infected cells are agglutinated by a non-protective antibody that is easily separated from an opsonising antibody associated with protection. Brown postulates that the agglutinating antibody serves to trigger a switch in antigens and this change allows the parasites to survive in what should have been an immune host. In other words this antibody has been manipulated by the parasite for its own ends. The opsonising antibody is important in protection and is effective against all antigenic variants and the relative rates of synthesis of these two antibodies determine the outcome of the infection.

The protective immune response to malaria is weak and largely ineffective and it is unlikely that any vaccine could produce a better immunity than the natural one. Nevertheless, acquired immunity to malaria does occur and children who survive the first few years of their lives in malarious areas tend to be immune to clinical malaria if not to the infection itself. A vaccine that could be used to see children through their first few years would be invaluable. There is no evidence to suggest that cell mediated responses are important in immunity to malaria so the induction of an antibody against merozoites could well be effective. Possibly all that is required is the antigen involved in binding merozoites to their host cells. As antigenic variation involves an antibody that has nothing to do with protection it is unlikely that antigenic variation would interfere with any protection produced as a result of vaccination against merozoites.

Correction

In the News and Views article "Biogenesis of surface membranes" (*Nature*, **249**, 414; 1974), Dr G. Kreibich's name was misspelled in the penultimate and last paragraphs.

Anthill and tiger counting in the Soviet Union

from Vera Rich

JUDGING from the numerous Soviet press and agency reports, one of the major tasks of Soviet ecologists and conservationists is the taking of censuses of various species of wild life. This applies not only to those species threatened by extinction, but of any that are or might prove of value to the economy. Thus the 1972 census of anthills in Estonia was undertaken, not because the Estonian ant was in imminent danger of disappearing, but because it had been found that an optimum of 3-6 anthills per hectare in stands of timber checks the multiplication of various forest pests. Similarly, the census currently being taken of sables in the Amur taiga will be used for improving the system of distributing hunting licences. Even in the case of threatened species, practical advantages are remembered: the Black Sea dolphin census (1971), which revealed that the dolphin population had doubled since the ban on hunting was introduced in 1965, evoked the remark from its director, A. Chepurnov, at a press conference: "The time is not far distant when dolphins will be used to drive fish into nets, to carry out marine rescue operations and to communicate with underwater laboratories".

The census-taking methods vary considerably in complexity. Counting anthills is a relatively simple task, which could conveniently be delegated to students as part of their practical work, but the estimation of most species is much more difficult. The latest (1973) Black Sea dolphin census, which gave a total of 800,000 (three times the 1965 figure), required aircraft and seagoing ships and took 3 months to complete. The grandiose estimation of the total dry-land flora of the world, carried out by the Botanical Institute of the Academy of Sciences of the USSR, giving a total 2,625,000 M tonne of phytomass, presumably involved satellite data. Sometimes a quantitative result seems impossible to obtain. In the case of the jeiran (a variety of antelope) of southern Tadzhikistan, although "large herds" are now reported where, before protection, only a few isolated specimens were observed, no figures, either absolute or relative, seem to have been established. Presumably, so far, no reliable method of counting them has been devised.

The same difficulty, it might be thought, would arise in the census of Amur tigers made from 1971-73 in the Primor'e region of the Soviet Far East, but an ingenious solution was found—

the investigators counted the tracks of tigers in the snow. Although at first glance this proposal seems to contain a source of confusion in the possible crossing and recrossing of its tracks by the same tiger, the photographs published (*Priroda*, No. 12, 82-88; 1973), show that although snow is not the ideal medium for revealing the fine details of the pad with fingerprint clarify, nevertheless, in practice, little or no confusion arises. Not only did this census give a total of some 110-120 tigers in the area (the figure for the 1940s was 30-40), but it revealed a number of significant details about the habits of tigers in the region.

Now that the Primor'e is being increasingly opened up for forestry, and the like, the tigers seem to be entering into a cautious symbiosis with man. (The Amur tiger is not a man-hunter; on the contrary, it is easily scared off by shouting and arm-waving.) The tigers make use of forest roads used for hauling timber, and seem to have no fear of tractors and automobiles. They appear, and may even hunt, relatively close to settlements and motor roads, and observe human activity from a distance of several metres. If disturbed after hunting, they will drop their prey, but are ready to return to it, even if it has been touched by human hand. And, since the presence of tigers causes a rapid disappearance of wolves from the area, the opening up of the Primor'e is in no way hampered by the protection of the tigers—indeed, their presence may prove an advantage. Providing, therefore, that no illegal tiger shoots take place—a condition which, it is admitted, is hard to guarantee—there seems no reason in principle why the tigers should not continue to flourish in these new conditions of coexistence.

Membrane proteins and serum lipoprotein

from a Correspondent

UNTIL recently, discussions of membrane structure centred around the concept of the unit membrane. The model envisaged was a lipid bilayer with hydrophilic proteins restricted to the outer surfaces where they were attached by electrostatic, non-covalent forces. Although this model was useful for explaining the similarities in physical and chemical properties between artificial lipid bilayers and natural membranes, it failed to account for all the proteins known to be associated with the membrane. This led several workers, notably Singer and Nicolson (*Science*, **175**, 720; 1972), to postulate that globular proteins penetrate into or through the lipid bilayer. This idea has been widely accepted as

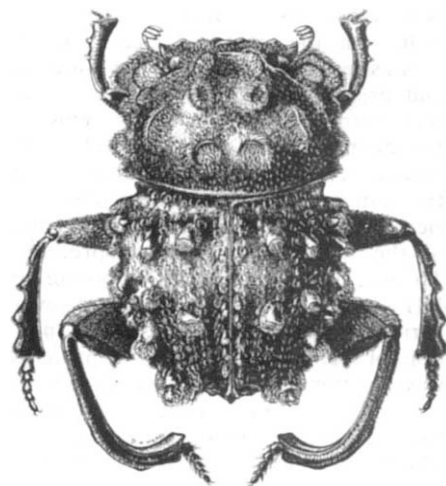
a sound working hypothesis, but the mode and distribution of the proteins in the plane of the membrane are still controversial. It has been convenient to divide membrane proteins into two groups; extrinsic proteins remain largely on the aqueous side of the membrane attached by electrostatic forces which can be disrupted by dilute salt solutions whereas intrinsic proteins make substantial contact with the hydrocarbon region of the lipid bilayer and can only be disrupted by use of ionic detergents. In practice, however, this distinction is difficult to apply to any protein because of the lack of structural information.

The human erythrocyte has occupied a central position in membrane research due to the ease of obtaining large quantities of membrane ghosts free of haemoglobin. By SDS gel electrophoresis, more than 20 membrane protein bands have been resolved (see, for example, Fairbanks *et al.*, *Biochemistry*, **10**, 2606; 1971) but, with few exceptions, the types and functional roles of the proteins have remained obscure. In particular, the presence of proteins with an apparent molecular weight in excess of 200,000 and apparently not dissociable into smaller subunits by treatment with thiols or detergents has been rather enigmatic. One would expect that intrinsic proteins would have more non-polar amino acids than do hydrophilic globular proteins, but differences have not been impressive. Furthermore, intrinsic proteins should have relatively short hydrophilic sequences capable of reacting with lipids. Glycophorin, the major glycoprotein of the human erythrocyte, contains a peptide chain of 203 residues which completely spans the membrane and has a portion of 23 residues devoid of charged groups (Segrest *et al.*, *Biochem. biophys. Res. Commun.*, **49**, 964; 1972); but the applicability of this situation to intrinsic protein in general cannot be assessed at present.

The erythrocyte membrane is not the only system where one can find lipids and proteins in close association readily accessible for extensive study. The circulating plasma lipoproteins are important sources of avidly bound lipids and proteins that are not membrane bound. In a recent issue of *Biochimica Biophysica Acta* (**342**, 213; 1974), Langdon now reports that the apoproteins of plasma lipoproteins are major constituents of the human erythrocyte membrane.

Langdon's evidence comes from both immunological and chemical analysis of the membrane proteins. Purified, packed erythrocyte ghosts were treated with sodium dodecyl sulphate to solubilise intrinsic proteins. Then the preparation was subjected to double

Tuberculated beetle



This strange-looking beast is a male dung beetle new to science. It has been named *Amphistomus primonactus* by Matthews of the South Australian Museum, Adelaide, who discovered it in the Dorrigo National Park. Its reduced hind body and exaggerated tuberculosity may be a consequence of wing reduction. It is inconspicuous and secretive, like the other wingless species of *Amphistomus*, and is abundant in closed forest leaf litter. It seems to have a very localised distribution (see *Aust. J. Zool.*, Suppl. Ser., No. 24, 1-211; 1974).

immunodiffusion on agar plates against antiserum to human density lipoprotein (apo HDL) and low density lipoprotein apoprotein (apo LDL). Strong precipitin lines indicated that the membrane proteins are related immunologically to both types of apoprotein (appropriate controls eliminated the possibility of non-specific precipitation). To determine whether the antigenic determinants resided in more than one molecular weight class, the membrane proteins were fractionated by Sephadex gel chromatography and examined by immunodiffusion. Although each protein class contained several proteins it was nevertheless noteworthy that antigenic determinants for both apo HDL and apo LDL antisera were present in all of them. Further tests showed that nearly half of the total membrane proteins are accounted for by the sum of the proteins reacting as the apo HDL and apo LDL and that probably antigenic determinants for both tested apoproteins are present in at least one protein molecule.

In order to fortify the immunological evidence with chemical evidence, end group analyses were performed. The membrane proteins were dansylated and hydrolysed and the resultant amino acids were analysed by quantitative two-dimensional polyamide chromatography. As expected, the major NH_2 terminals were aspartic

and glutamic acids, characteristic of apo HDL and apo LDL, respectively. Together they constituted 40% of the NH₂ terminals, a fact which agrees well with the immunological data, if it is assumed that most of the glutamic and aspartic acids are present as the NH₂ terminals of the apoproteins in the membrane. COOH terminals were determined by standard techniques and the results showed that these too were rich in terminal residues characteristic of the serum lipoprotein apoproteins. To discover whether the apoprotein NH₂ terminals were distributed over various molecular weight sizes, a portion of dansylated membrane proteins was fractionated by SDS electrophoresis and the amino acids from each band chromatographed on polyamide. The results showed that the characteristic NH₂ terminals of serum apoproteins were distributed over a wide range of molecular sizes, in agreement with the immunological data.

The pattern of the NH₂ terminal amino acids together with certain of the immunological data and the high molecular weights observed led Langdon to consider that the apoproteins may be linked in the membrane to form larger molecules. Previous work had shown that disulphide links are not present in membrane proteins so Langdon investigated the possibility of lysine-derived crosslinks, more usually found in connective tissue (*Biochem. biophys. Acta*, **342**, 292; 1974). He indeed found positive evidence for such links using chemical techniques similar to those used for examining proteins like collagen and elastin.

Langdon's data pose some intriguing and important questions. Are serum lipoprotein apoproteins the major intrinsic proteins of the erythrocyte by virtue of their affinity for lipids? And do they form a structural basis for the covalent attachment of enzymes and other protein types? Are the apoproteins synthesised in the haemopoietic system during erythropoiesis or are they derived from circulating lipoproteins? If Langdon's results are confirmed, they could have far-reaching implications in both membrane biochemistry and in cardiovascular research.

Functions of the cell periphery

by Peter Newmark

By shrewd invention of a title the organisers of a discussion meeting held at the Royal Society on June 19–20 managed to attract a diverse range of speakers on the pericellular environment and its regulation in vertebrate tissues. Within this framework many topics were touched on although most

contributions were concerned with connective tissue cells and matrix.

The problems of understanding the metabolism of the matrix are based partly on the complexity of its component proteoglycans, glycosaminoglycans and tensile fibres and partly on separating the synthetic and degradative parts that connective tissue cells play in the turnover of the matrix. H. Muir (Kennedy Institute for Rheumatology, London) reported on the disproportionate importance of the minute amounts of hyaluronic acid recently shown to be present in cartilage matrix. It seems that the distinct proteoglycan aggregates found there are formed by virtue of the affinity of one end of the proteoglycan's core protein for multiple sites on the hyaluronic molecules. An additional important role for hyaluronic acid in cartilage is implied by the demonstration that it inhibits the production of proteoglycans by chondriocyte suspensions, possibly by means of a cell membrane effect.

The role of the membrane in matrix turnover was also raised by the studies of J. T. Dingle (Strangeways Research Laboratory, Cambridge). In addition to reporting on two new cathepsins that may play a part in proteoglycan breakdown, he also elegantly demonstrated that cathepsin D is localised on or around the cell membrane during its stimulated secretion from cells. A functional importance of this phenomenon was suggested by it also being demonstrated in cartilage taken from human rheumatoid arthritic joints. The importance and site of action of the cathepsins, all of which have an acidic pH optimum, however, remain to be elucidated.

P. Davies (Clinical Research Centre, Harrow) drew parallels between the development of glomerular nephritis and asbestosis. The activation of lysosomal enzymes by immune complexes in the first case and by asbestos particles in the second disease can result in the breakdown of the basement membrane. This, perhaps with contributory mechanisms, can trigger off the overproliferation of epithelial cells and thickening of the basement membrane that is associated with both diseases. Cell proliferation, in this case of the arterial smooth muscle cells, was also one of the topics discussed by R. Ross (University of Washington). When grown in culture, these cells, like so many others, only proliferate in the presence of serum. It seems, however, that the important growth factor(s) is initially derived from platelets. The importance of this factor could be that, when released from platelets at a site of arterial injury, it causes the local proliferation of subendothelial cells in atherosclerosis.

In a speculative vein J. E. Scott

(MRC Rheumatism Unit, Taplow) contemplated the evolutionary forces that may have determined the universality of polyanionic carbohydrates in cell membranes and matrix. In prebiotic times they would have produced an excellent shield against radiation-produced hydrated electrons; with the advent of aerobic conditions they would still be very stable; and, finally, they possess excellent properties for withstanding the wear and tear to which extracellular molecules are subjected.

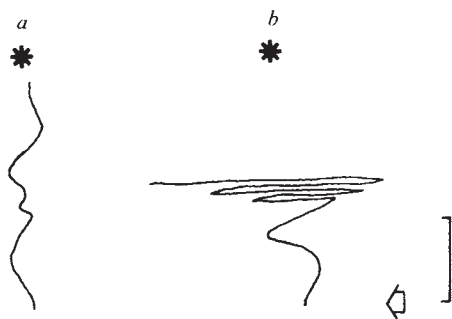
In his continuing studies of cell-cell recognition, M. M. Burger (University of Basel) has recently turned his attention to a model system, the sponge. Suitable treatment of sponge cells results in their releasing an aggregation factor and a base plate. If the latter is covalently attached to cell-sized solid beads, the addition of the aggregation factor and calcium results in bead aggregation. This neat system promises to assist in the elucidation of the molecular basis of cell interactions.

Orientation to odours by insects

from our Insect Physiology Correspondent

It has long been known that insects fly upwind to attractive odours—mosquitos finding their hosts, drones locating the queen bee, fruit flies seeking fermenting fruit—and that the maintenance of the direction of flight is a visual response to the pattern of the ground below. In other words that anemotaxis in the flying insect is an optomotor response. But a year or two ago (*Science*, **173**, 67; 1972; **180**, 1302; 1973) Farkas and Shorey claimed that in response to the sex pheromone of the female, flying males of the pink bollworm moth *Pectinophora* are guided by a chemotactic mechanism which can operate in still air. The existence of such a mechanism of oriented flight in the absence of a moving air stream (as opposed to a kinetic mechanism dependent on gradients in the concentration of odours, or a visually oriented mechanism in an air stream) could be disturbing for accepted theories of orientation by flying insects.

Kennedy and Marsh now point out (*Science*, **184**, 999; 1974) that in the experiments of Farkas and Shorey the males were already in flight and may already have been oriented anemotactically before the air stream was stopped. They have therefore repeated the experiments on males of the dried fruit moth *Plodia interpunctella* and related moths; the movements of the insects were accurately recorded in a wind tunnel and the results were analysed statistically. The flight of male insects in a stream of air in a wind tunnel could be completely controlled by the



Two 3 s flight tracks (based on videotape records) of the same male *Cadra cautella* after its arrival by upwind flight at a point 0.50 m from a source of female scent. To the left (a): the source remained throughout the upwind position marked by the star. To the right (b): the source was removed as soon as the male reached the level of the arrowhead so that the male entered unscented air after travelling approximately 0.15 m further upwind. (Scale: 0.15m.)

movement of black and yellow stripes on the floor. If the stripes were stationary the males flew upwind against the stream of odour from the female; if the stripes moved downwind above a certain velocity the flying male also was carried downwind. If the odour was suddenly removed while the male was in flight, but the odourless air stream continued, the male arrested its upwind flight and flew at right angles to the air stream, casting to and fro over a progressively widening band (see figure). This response was unchanged if one antenna was removed, which would not have been the case if a chemotropotactic mechanism were involved.

It seems, therefore, that an odour-controlled optomotor anemotaxis is still the most plausible mechanism to account for the response of the male moth to the sex pheromone of the female; and that the side to side casting when the odour trail is lost, which had been noted in the past, is an important element in the optomotor reaction.

Anchoring the Ig molecule

from a Correspondent

It is generally accepted that immunoglobulin (Ig) acts as the antigen recognition unit at the surface of bone marrow-derived (B) lymphocytes. The chemical homology between the membrane-bound Ig and the molecules secreted by the activated antibody-forming B cell is unknown although this must be considerable since the surface-bound Ig component can be detected if serum reagents specific for both variable and constant regions are used. This raises the problem of how a soluble Ig glycoprotein molecule is

adapted to perform a function which requires firm attachment to the cell surface membrane. In many integral membrane proteins and glycoproteins a small hydrophobic segment of peptide anchors the rest of the molecule into the lipid bilayer of the membrane. It is possible certainly that the polypeptides of surface Ig contain an extra stretch of amino acids suitable for this purpose. If the two or three well studied examples of membrane proteins are considered, these amino acids would be located at the carboxyl terminal of heavy chains in the constant (Fc) portion of the molecule.

An alternative method for anchoring an essentially water soluble component to a membrane involves attachment to an integral membrane component with a recognition site for some part of the soluble protein. This method is favoured by Ramasamy *et al.* (*Nature*, **249**, 573; 1974) for surface-bound Ig.

B lymphocytes as well as macrophages, mast cells and polymorphonuclear cells possess surface receptors for the constant (Fc) portion of Ig. It is proposed that these receptors tightly bind Ig molecules to the B cell surface in such a way that the antigen binding sites of the variable region of Ig extend into the extracellular space and are available to specific antigen. The murine plasma cell tumour MOPC 21 (P3) secretes IgG1 kappa molecules in culture, a small proportion of which may be assumed to bind to and saturate the putative Fc surface receptors. Indeed, no Fc receptors are detectable on these cells. Mutants blocked in Ig secretion, however, can be obtained and these also carry no surface Ig molecules. Ramasamy *et al.* argue that in these cells the Fc surface receptors should be demonstrable and this proved to be the case. That is to say there is in general an inverse correlation between the presence of surface Ig and Fc receptors.

The surface Ig of mouse B lymphocytes is probably monomeric IgM, and it seems that the secreted pentameric IgM may have decreased affinity for Fc receptors since secreted IgM only weakly inhibits Fc rosettes on B cells. The interaction of Ig with Fc receptors also seems in some cases to require intact Ig carbohydrate units. Williams *et al.* (*J. Immunol.*, **111**, 1690; 1973) have treated rabbit IgG preparations with an endo- β -N-acetylglucosaminidase from *Diplococcus pneumoniae*. About a half of the carbohydrate, present largely in the Fc portion of the Ig molecule, is removed. The bacterial agglutinating activity of anti-pneumococcal IgG was unaffected by this treatment whereas complete loss of opsonic activity occurred. Similarly several treated IgG preparations had markedly decreased ability to inhibit

rosette formation between human monocytes (carrying surface Fc receptors) and IgG-coated erythrocytes. Not all IgG preparations were affected in this way, however, (for example, anti-streptococcal), although similar amounts of carbohydrate were removed by the glycosidase.

The reason for this finding is not clear. Since it cannot yet be excluded that the detailed carbohydrate structure of Ig molecules may differ according to species or possibly with the particular cell clones activated to produce antibody, it seems likely that the type of carbohydrate structure left behind on the treated Ig molecule may also vary. In some cases the biologically relevant sugar sequences might not be removed by glycosidase treatment. Further work using additional enzymes will be required to remove more than half of the carbohydrate, leaving the Ig polypeptide chains intact. Only then will it be feasible to decide if significant differences in the mechanisms for opsonisation and phagocytosis of different bacteria exist rather than the simpler explanation that differences in carbohydrate structure occur in Ig molecules of different specificities.

Terminal sequences of animal virus RNAs

from Alan E. Smith

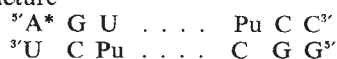
To date, attempts to sequence the RNA of animal viruses have lacked the spectacular success of bacteriophage RNA sequence work. This is largely because of the difficulties encountered with animal viruses in obtaining either highly labelled RNA for analysis by the Sanger technique or large amounts of RNA for sequence determination by more classical methods. Nevertheless, methods are being developed to overcome these problems and among the most successful are the techniques for the enzymatic introduction of radioactive phosphate into specific positions within viral RNA molecules.

Silkworm cytoplasmic polyhedrosis virus (SCPV) contains ten pieces of genomic double-stranded RNA, and in this and other respects it is very similar to the more familiar reoviruses. Some time ago the nucleotides present at the ends of reovirus double-stranded RNA were determined by isolating 32 P-labelled viral RNA and subjecting this to Sanger RNA sequencing techniques. All ten fragments of reovirus were found to have a 5' terminal guanosine 5' diphosphate followed by a pyrimidine residue (*J. molec. Biol.*, **61**, 643-653; 1971) and a 3' terminal cytosine (*Nature new Biol.*, **232**, 114-115; 1971). Each genome fragment therefore

seems to have a perfect duplex form.

In a recent issue of the *Journal of Molecular Biology* (85, 31–48; 1974) Miura, Watanabe and Sugiura report a similar analysis of silkworm cytoplasmic polyhedrosis virus. To avoid the difficulties in labelling silkworms with ^{32}P to produce radioactive virus, the isolated viral RNA was treated with phosphomonoesterase to remove any 5' phosphates and then labelled in the 5' OH position using polynucleotide kinase and $[\gamma^{32}\text{P}]$ ATP. The native double-stranded RNA molecules proved very difficult to label by this method, however, and to achieve efficient labelling Miura *et al.* first removed chemically two or three residues from the 3' end of the molecules, thus exposing a short single-stranded 5' terminal region. Presumably such single-stranded tails are much more accessible to enzymatic modification. The labelled molecules were then analysed by ribonuclease digestion followed by column chromatography. Two 5' termini were found and were identified as A*GU and GGC. The identity of the 5' terminal base A* is as yet uncertain but it is probably an adenosine residue with a modified 2' OH group. Such modified bases, although a common feature in transfer RNA molecules, are rare in viral RNAs. The 5' terminal sequences are consistent with perfect duplex formation in the SCPV genome since the same authors have previously identified cytosine and uridine as the 3' terminal bases.

To determine whether all of the double-stranded genome segments contain both 5' terminal sequences or if some fragments contain symmetrical ends, all of the ten genome segments were separated on polyacrylamide gels and then individually subjected to end-group analysis. This showed that every fragment contains both sequences and suggests that each has the structure



As with reovirus, silkworm cytoplasmic polyhedrosis virus particles contain an RNA-dependent RNA polymerase which actively transcribes the double-stranded RNA into messenger RNA *in vitro*. All ten mRNA molecules made *in vitro* by the reovirus enzyme begin with ppG (Banerjee, Ward and Shatkin, *Nature new Biol.*, 230, 169–172; 1971). Further sequence data on highly labelled mRNA made from the smaller double stranded RNA segments has been presented by Nichols, Hay and Joklik (*Nature new Biol.*, 235, 105–107; 1972). Some 5' terminal guanosine triphosphate as well as diphosphate was detected in this study, but no protein synthesis initiation codon was present within the first 25

A larger Gondwanaland?

from Peter J. Smith
Geomagnetism Correspondent

ALTHOUGH the principal features of the ancient supercontinent of Gondwanaland are now widely accepted, there are still some details (if whole countries may be so regarded) and disagreements to be settled. One outstanding problem, for example, concerns the present position of the continental crust which once lay between Australia and India. Somewhat less of a problem in many people's eyes is the nature of the present join between India and the rest of Asia. Dewey and Bird (*J. geophys. Res.*, 75, 2625; 1970) suggested that the Himalayas resulted from continental collision; and such has been the influence of the article in which this proposal appeared that some of the difficulties with this interpretation have perhaps not been given the attention they deserve.

At least, that seems to be the view of Crawford (*Science*, 184, 1179; 1974) who cites Meyerhoff's claim (*J. Geol.*, 78, 1; 1970) that there is evidence against India's colliding with the rest of Asia. Certainly, there are problems here, which is why Powell and Conaghan

(*Earth planet. Sci. Lett.*, 20, 1; 1973) recently proposed that although the Himalayan mountain chain is basically of the collision type, it was not formed as a direct result of the collision. But Crawford, who at the time of writing was apparently unfamiliar with the Powell-Conaghan model, goes further in proposing that the Himalayas formed intracontinentially and arguing against collision on the grounds that the Indus Suture Line, "if it is the relic of oceanic subduction preceding collision, lies on the wrong side of the Himalayas".

In Crawford's reconstruction, Gondwanaland was originally larger than is usually supposed and included Tibet, the Tarim Basin block and parts of northern China. It was Tibet, in fact, which lay between India and western Australia in the form of submerged continental crust. The Indus Suture Line is then seen as "a relic of a Permojurassic oceanic opening to the mantle" which closed at the end of the Jurassic. The Himalayas later developed along fractures which had formed parallel to the opening of the Indus Suture Line.

5' terminal residues sequenced.

Shimotohno and Miura (*J. molec. Biol.*, 85, 21–30; 1974) now report that the 5' sequence of the RNA made by the silkworm virus enzyme is ppAGPy, and this unique sequence is present in the mRNA transcribed from each of the genome fragments. This indicates that transcription to make mRNA begins at the first base of each molecule, reading from the strand with the 3' OH sequence PuCU. Perhaps the unusual base at the 5' end of the complementary chain plays some part as a recognition site for the binding of the polymerase molecule.

It would be interesting to determine whether the 5'-terminal A in mature mRNA is modified, since the presence or absence of such a modification might be used to distinguish between molecules destined to be messengers from those making up progeny genomic RNA. So far studies on reovirus multiplication have shed no light on the distinction between transcription to give mRNA and replication to give double-stranded RNA, nor on how they are controlled relative to one another. Apparently, the presence of poly (A) in the mRNA species cannot be used as a distinguishing feature since reovirus mRNA does not contain this appendage (*J. biol. Chem.*, 248, 7993–7998; 1973).

Precursors of strike-slip faulting

from Peter J. Smith
Geomagnetism Correspondent

THE travel-time residuals of seismic waves—the differences between observed arrival times and the corresponding arrival times computed using a standard Earth model—are due (in addition to random experimental errors) partly to imperfect source determination and partly to differences in velocity between the real Earth and the assumed model. But if at a particular seismograph station the residuals from many sources at many different locations are averaged, the random errors associated with particular wave paths and particular sources should cancel out, thereby making it possible to isolate the common part of the residuals arising from velocity deviations in the crust beneath the station. If the seismic events concerned are also well distributed in time, it should then be possible to determine any secular variations in the near-station contribution to the residuals, and even to determine changes in the near-station crustal velocity (given the source volume).

Applying this technique to 3,000 P wave residuals for events in the Matsushiro area of Japan during the period 1960-1968, Wyss and Holcomb (*Nature*, **245**, 139; 1973) were able to show that the Matsushiro series of earthquakes of 1965-1966 had been preceded by an anomalous decrease in P wave velocity in the source region. Specifically, the P wave residuals began to increase significantly from the long term average of -1.25 s around October 1962 (about 3 years before the earthquake series began), representing a decrease in P wave velocity near the Matsushiro station of about 20% (assuming a source volume with a radius of about 16 km). The residuals then returned to within one standard deviation of the long term average about 330 d before the Matsushiro swarm began and about 770 d before the swarm's principal energy release.

At the time that Wyss and Holcomb were carrying out their analysis they were apparently aware that premonitory changes in seismic velocity in earthquake source regions had already been observed in the Soviet Union, New York State and the Transverse Ranges of southern California; and so in that sense they were neither reporting a new phenomenon nor pointing out for the first time its possible application to earthquake prediction. On the other hand, the Matsushiro result was more than just another example of a known effect, for it was the first time that premonitory velocity changes had been observed before strike-slip faulting.

Not the least interesting aspect of this discovery was that, at about the same time, several groups of workers were attempting, and apparently failing, to find comparable premonitory changes along the active central section of the San Andreas fault—and Nur *et al.* (*Stanford Univ. Publs. geol. Sci.*, **13**, 391; 1973) had even suggested that the explanation for velocity changes in terms of dilatancy may not be applicable generally to strike-slip faults.

But all that has now changed, for Robinson *et al.* (*Science*, **184**, 1281; 1974) report that, using P wave residuals as indicators, they have been able to observe changes preceding the Bear Valley earthquake of February 24, 1972 (surface magnitude, 5.0). The travel-time residuals analysed were from small local earthquakes which occurred along the Calaveras fault north-west of the Bear Valley region between June 1, 1971 and May 30, 1972 and which had been located precisely using the US Geological Survey's network of seismograph stations, excluding the three station (BVL, which lies almost above the focus of the Bear Valley earthquake, and the nearby EKH and JHC) at which the residuals them-

selves were observed. In all, 49 shocks were observed sufficiently well at BVL and a slightly smaller number at each of the other two stations.

The expected range of 'normal' residuals about their mean value was ± 0.15 s; and for most of the one year observation period, residuals were indeed within these limits. But between late December 1971 and late January 1972 the residuals at BVL were anomalously high at about 0.3 s above normal values and, in the statistical sense, significantly higher on average than the mean for the preceding 7 months. As the eight events within the anomalous period covered a distance range of 50 km (20-70 km), a magnitude range of 2.7 (0.7-3.4) and a depth range of 7 km (2-9 km), and were apparently no different in such respects from the other events, there seems little reason to doubt that their anomalous nature represents a genuinely precursory indication of the February 24, 1972 earthquake.

Unfortunately, data from the other two stations were ambiguous, with only two of the seven residuals within the December-January period at EKH and none of the five corresponding residuals observed at JHC lying outside the 0.15 s limit, and so to that extent confirmation was lacking. But accepting the BVL results, Robinson and his colleagues conclude that the anomalous changes in residuals are equivalent to a 10-15% decrease in P wave velocity within a volume having the same radius (7 km) as the observed aftershock zone. This decrease began 60-53 d before the Bear Valley shock itself, which is consistent with the time scale deduced for a magnitude 5.0 event using other methods. And as seems to be usual in such circumstances, the velocity returned to its normal value before (in this case several weeks before) the onset of the main event, although the data are not sufficient to indicate whether this return was sudden or gradual.

Naturally, this result raises the interesting question of why other workers (using different methods) have failed to observe premonitory changes, even when investigating the same earthquake: McEvilly and Johnson (*Science*, **182**, 588; 1973) for example, examined the stability of P wave travel times along a path passing about 10 km north of Bear Valley, and concluded that the 1972 Bear Valley shock was preceded by no significant changes. In this case, however, failure seems to have been simply a matter of bad luck. McEvilly and Johnson made use of quarry blasts as sources; but unfortunately, as Robinson *et al.* point out, no blast actually took place during the anomalous period now recognised from the BVL residuals.

Octupole states in cadmium

from Peter E. Hodgson
Nuclear Theory Correspondent

THE energies, spins and wavefunctions of low lying nuclear states can often be calculated from a simple model, and it then becomes particularly interesting to see if they can be found experimentally. If some of them are predicted by one model but not by another, they become important for deciding which of the models is to be preferred.

An example of such a state is the 3^- octupole state expected on the collective vibrational model at about 2 or 3 MeV in heavy nuclei. Such states had been found in the even isotopes of cadmium, and this was accepted as evidence for the collective nature of these nuclei.

Collective states are strongly excited by inelastic scattering. The energies of the inelastically scattered particles give the energies of the nuclear states, and their angular distributions and intensities give the quantum numbers and strengths of the states. In the case of vibrational nuclei the strength of a state is related to the dynamic deformation. The work on ^{116}Cd showed a 3^- state at 1.9 MeV with a strength similar to that expected from the collective model.

This has now been challenged by some accurate new measurements by Gill and colleagues at the University of Alberta (*Phys. Rev. Lett.*, **32**, 889; 1974) of the energies of the γ rays emitted after the inelastic scattering of neutrons by ^{116}Cd . As shown in Fig. 1, they found γ rays of 1,404, 1,409 and 1,416 keV, corresponding to the decay of states at 1,917, 1,923 and 1,930 keV to the state at 513 keV. None of these states decays to the ground state to a detectable degree. Thus, what was previously interpreted as a single 3^- state is now resolved into three separate states with quite different properties. As shown in Fig. 2, the sum of the inelastic scattering cross sections to these three states is similar to that expected from a single

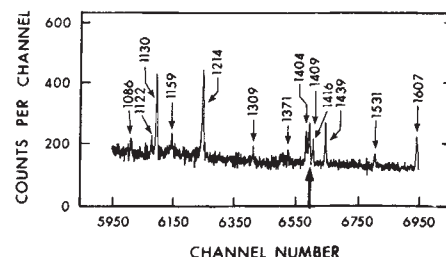


Fig. 1 Spectrum of γ rays after the inelastic scattering of neutrons by ^{116}Cd , showing the line at about 1,410 keV resolved into three peaks.

3^- state, but individually they correspond to 0^+ states. Thus the evidence previously interpreted as indicating a single 3^- state is now re-interpreted in quite a different way. There is no other state in the required energy region that could plausibly be interpreted as the required 3^- collective state, so the evidence for the collective nature of this nucleus is weakened.

This result is important for the understanding of the structure of cadmium, but it has several wider implications. First, it is a reminder of the importance of precision in physical measurement. Time and again increased precision does not merely sharpen the picture, but reveals wholly unexpected details that oblige one to adopt a completely new interpretation. Second, it is a reminder of the provisional nature of much of the day-to-day details of scientific work, particularly on the frontiers of knowledge. In so many cases scientists build ideas on quite flimsy and scattered evidence; it is right for them to do this provided they realise how subject it is to revision in the light of new information.

It is never sufficient to be satisfied with an interpretation that explains the incomplete set of experimental data available. Every consequence of an interpretation needs to be tested as rigorously as possible, and even then one must be prepared for the new unexpected result that calls it all in question again.

Acceptable radiation exposure

from J. R. A. Lakey

THE chairmen of both the International Commission on Radiological Protection (ICRP) and its counterpart on Radiation Units and Measurements (ICRU) were opening speakers at the Society for Radiological Protection International Symposium held at Aviemore, Scotland from June 2 to 6. These two commissions provide the ground rules for all aspects of radiological protection and both place considerable stress on the measurement of radiation and its interpretation.

C. G. Stewart (ICRP) opened the symposium with a statement on the ICRP recommended Investigation Levels and Derived Working Limits and described these as signposts to direct action which could also be used as criteria for discarding unnecessary information. Radiation exposures exceeding these levels demand more careful consideration and higher accuracy of measurement. J. Dunster (National Radiological Protection Board, Harwell) endorsed this thesis and said that errors of a factor 2 are acceptable in some radiation measurements but this does not excuse lack of clarity in thought on the part of the health physicist.

P. J. Campion (National Physical Laboratory, Teddington) described metrology as the cornerstone on which all advances in radiological protection could be built. In addition to the necessity for instrument calibration he stressed the need for a strict hierarchical structure so that the calibration of a particular instrument could be traced back to the national standard. R. Maushart (B. F. Vertriebes, Karlsruhe) appealed for standardisation of the methods of using instruments so that calibration is not an end in itself.

H. L. Wyckoff, the chairman of the ICRU (Washington DC) presented an elegant resumé of the quantity of dose equivalent which has been jointly developed by his commission and the ICRP. This quantity is expressed in the rem unit, derived from the absorbed dose with corrections depending on the nature of the radiation.

R. H. Mole (MRC Radiobiology Unit, Harwell) agreed that a relation-

ship must exist between the absorbed dose of radiation and the degree of probability of an effect on the exposed person. Philosophical problems emerge when risks to population are computed by the summation of risks to individual members. It is particularly difficult to place these computed population risks into perspective and a great deal has yet to be learned about public acceptance of these estimates. On the other hand the concept of risk or detriment to an individual organ of the body is useful for the summation of radiation exposure to the body due to intake of a single radionuclide. J. Vennart (MRC Radiobiology Unit, Harwell) said that this estimate of risk could be derived from the forthcoming ICRP recommended Maximum Permissible Annual Intake which is to replace the less satisfactory Maximum Permissible Body Burden. He said that the commission would also publish Derived Air Concentrations but there was to be no attempt to give limits for drinking water for occupationally exposed persons, who, after all, consume the same water as the public.

The legal attitude to radiation protection varies between countries to the extent that the radiation worker could be restricted in mobility and so the European Economic Community aims to improve this situation through its directives to member countries. These proposals, summarised by P. Recht (EEC), are expected to involve some changes in United Kingdom legislation in spite of the common ICRP basis. A. W. Kenny (Department of the Environment, London) also showed that the EEC would become a new factor in the control of radioactive waste. The UK approach presented by A. Preston (Fisheries Laboratory, Lowestoft) is to limit radioactive waste disposal by restricting radiation exposure to members of a critical population group. The main difficulty in the application of this procedure is the need to find a completely objective method of selecting the critical group. W. D. Rowe (US Environmental Protection Agency, Washington DC) described an alternative approach which makes use of comprehensive mathematical models which include source terms, environmental transport pathways and human metabolism to predict the dose to members of the public. These models require some form of measurement for their periodic validation but might avoid some of the problems of the critical group method.

Preprints of most of the papers which were presented can be obtained on payment of a small fee from Mr K. B. Shaw, National Radiological Protection Board, Harwell, Didcot, Berkshire OX11 0RQ.

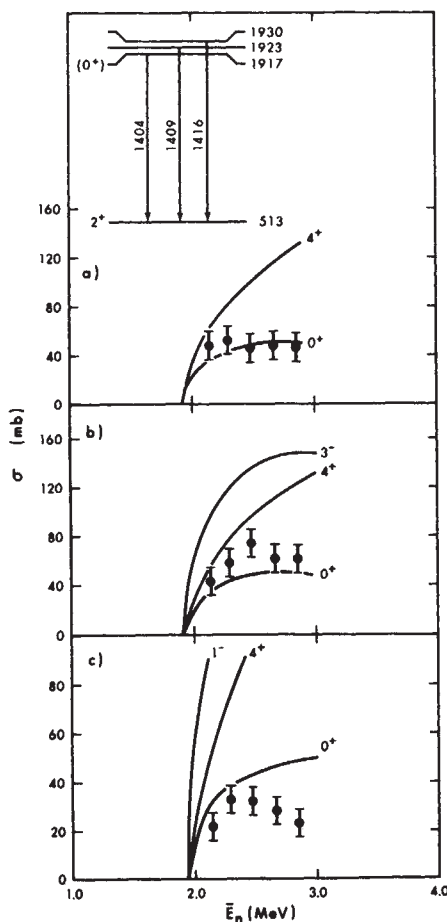


Fig. 2 Excitation functions for the total cross sections for the excitation of the states of 1,917, 1,923 and 1,930 keV by inelastic neutron scattering. The sum of the individual intensities is compatible with 3^- , but individually they cannot be 3^- and are likely to be 0^+ .

Chronological and ecological implications of the fossil Bovidae at the Sterkfontein Australopithecine site

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Bovid remains indicate the presence at Sterkfontein of three faunal phases, accumulated at different times and probably under different climatic conditions. These phases are variously associated with such well known Sterkfontein finds as the type locality australopithecine assemblage, the West Pit (extension locality) hominid remains which may include Homo and numerous stone artefacts.

SEVERAL hundred bovid cranial fragments, mostly dentitions, hailing from the various localities at the Sterkfontein excavation site (see Fig. 1), were available for this study. Associated with the famous australopithecine material at the Sterkfontein main quarry (type locality—henceforth referred to as STS)—were 111 specimens. Forty-two specimens came from the West Pit (extension locality, SE), which was excavated by J. T. Robinson in 1957 and 1958 (ref. 2). Some 200 specimens came from rubble dumps D1, D2, D3, D5, D6, D8, D12-16 and H2, excavated by P. V. Tobias and A. R. Hughes, who placed these materials at my disposal for study. These dumps, STS and SE will be referred to throughout this work as site units. The bovid species identified at Sterkfontein are shown in Table 1.

Of primary interest in this study is what information the Bovidae can provide about a possible time difference between the two main hominid-bearing site units, STS and SE. It has been suggested that SE may belong to a later time period than STS. Robinson² wrote of the presence at Sterkfontein of an earliest Lower breccia including STS, which he believed to be unconformably overlain by a later Middle breccia including SE. He also recognised a third, youngest Upper breccia. Tobias⁴ felt that unlike STS, the SE assemblage includes at least a trace

of a hominid more advanced than *Australopithecus*. An important contribution to any discussion of the affinities of STS and SE is the fact that many stone tools have been recovered from SE. They were first discovered there by Brain⁵, and have since been found in numbers totalling hundreds at SE and in several dumps. None has been found in association with *Australopithecus* at STS. The first published suggestion that some major South African fossil assemblages can be arranged in a succession of Faunal Stages (this was later amended to Faunal Spans⁶) came from Wells⁷, who included STS in the earlier Sterkfontein stage and SE in a later Swartkrans stage. This is the first time that Sterkfontein faunal remains have been included in the

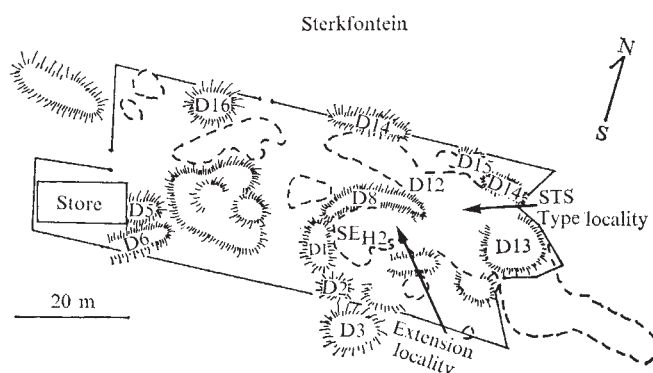


Fig. 1 Plan of the Sterkfontein Excavation Site (after Tobias and Hughes¹). SE, West Pit; D1-16, rubble dumps; H2, material excavated under SE; - - - caves, holes and excavations.

Table 1 Minimum numbers of individuals in bovid species at Sterkfontein. Taxonomy of extant Bovidae as in ref. 3; E, extinct; IR, indistinguishable from recent; 'larger small' as in my unpublished work

	Species	STS	SE	H2	D1	D2	D3	Site units		D5	D6	D8	D12	D13	D14	D15	D16
1	<i>Syncerus cf. acoelotus</i>	E	1														
2	<i>Taurotragus cf. oryx</i>	IR	1											2			1
3	<i>Tragelaphus cf. strepsiceros</i>	IR										2					
4	<i>Tragelaphus sp. aff. angasi</i>	E	1														
5	<i>Tragelaphus cf. scriptus</i>	IR															2
6	<i>Pelea cf. capreolus</i>	IR															3
7	<i>Redunca cf. arundinum</i>	IR	1														
8	<i>Kobus cf. ellipsiprymnus</i>	IR			1												
9	<i>Hippotragus cf. equinus</i>	IR	2														
10	<i>Hippotragus cf. niger</i>	IR									1						4
11	<i>cf. Hippotragus sp. aff. gigas</i>	E	8	1										5	1	1	
12	<i>cf. Megalotragus sp.</i>	E	1														
13	<i>Connochaetes taurinus</i> lineage	E + IR	1	3								1		1			4
14	Medium-sized alcelaphines	E + IR	7	6	2	2		1									1
15	<i>Damaliscus sp.</i> ('larger small')	E	7	3	2	2		2				1	1	2			5
16	<i>Damaliscus cf. dorcas</i>	IR		3			1	2				1					11
17	<i>Antidorcas cf. recki</i>	E	2	3													
18	<i>Antidorcas cf. marsupialis</i>	IR			1							1					2
19	<i>Antidorcas bondi</i>	E	1	2	1	1	1					3					5
20	<i>cf. Gazella vanhoepeni</i>	E	1											2			
21	<i>Oreotragus major</i>	E		1										1			
22	<i>Ourebia cf. ourebi</i>	IR										1					3
23	<i>Raphicerus cf. campestris</i>	IR															2
24	<i>Makapania cf. broomi</i>	E						1									
Minimum numbers of individuals of all species			42	22	8	7	1	1	6	1	10	1	17	1	2		40

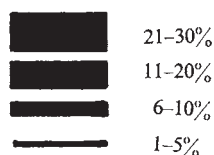
discussion of the contemporaneity or otherwise of STS and SE.

To date there has been no published attempt to relate the fossil content of the rubble dumps to the *in situ* breccia of Sterkfontein. If the Sterkfontein fauna was really assembled during successive time periods, whether in a series of breccias as visualised by Robinson or as part of a continuous breccia,

it has to be concluded that the contents of the rubble dumps may be mixed with respect to such time periods. Nonetheless any one dump should contain a high proportion of fossils from a single part of the deposit, and so from a single time period. An attempt is made here to group these dumps, on the basis of their bovid fossil content, with each other and with STS and SE.

	Faunal phase A	Faunal phase B	Species also present (X) or probably present (?X) at Makapans- gat Lime- works
	STS	SE	
<i>Hippotragus</i> cf. <i>equinus</i> (IR roan antelope)			
cf. <i>Megalotragus</i> sp. (E large hartebeest type)	-----		
<i>Redunca</i> cf. <i>arundinum</i> (IR reedbuck)			?X
<i>Syncerus</i> cf. <i>acoelotus</i> (E buffalo)			?X
<i>Tragelaphus</i> sp. aff. <i>angasi</i> (E nyala)			?X
cf. <i>Gazella vanhoepeni</i> (E gazelle)			X
<i>Makapania</i> cf. <i>broomi</i> (E muskox type)	██████████	-----	X
cf. <i>Hippotragus</i> sp. aff. <i>gigas</i> (E large hippotragine)	██████████	-----	X
<i>Antidorcas</i> cf. <i>recki</i> (E springbok type)		██████████	
<i>Antidorcas bondi</i> (E springbok type)	-----	██████████	
<i>Oreotragus major</i> (E klipspringer)			X
<i>Taurotragus</i> cf. <i>oryx</i> (IR eland)			
<i>Damaliscus</i> cf. <i>dorcas</i> (IR blesbok)		██████████	
<i>Damaliscus</i> sp. 1 or <i>Parmularius</i> sp. (E blesbok type)	██████████	-----	
<i>Damaliscus</i> sp. 2 (E blesbok type)	-----	██████████	
<i>Connochaetes taurinus</i> lineage (E and IR? blue wildebeest)			?X
Medium-sized alcelaphines (E and IR)	██████████	██████████	?X
Total number of cranial and dental specimens	111	42	
Total minimum number of individuals	42	22	
Number of extinct : indistinguishable from recent species	12 : 2	8 : 3	
% constituted by minimum number of definitely extinct individuals of total minimum number	$\frac{39 \times 100}{42 \times 1} = 93\%$	$\frac{17 \times 100}{22 \times 1} = 77\%$	
% constituted by minimum number of alcelaphines and antilopines of total minimum number	$\frac{20 \times 100}{42 \times 1} = 48\%$	$\frac{18 \times 100}{22 \times 1} = 82\%$	

Line thicknesses represent minimum number percentages per species per site as follows:



----- Only one or two specimens present which are suspected of belonging to a different faunal phase

Fig. 2 Distribution of bovid species between STS and SE. The specimens here represented are only those deriving from *in situ* STS and SE breccia. *Damaliscus* sp. of Table 1 and Fig. 4 is divided into *Damaliscus* sp. 1 or *Parmularius* sp., and *Damaliscus* sp. 2; nearest extant relative given in each case; E, extinct; IR, indistinguishable from recent.

Statistical methods and results

In site units STS and SE species, as represented in Table 1, were separated into five tribes or tribal groupings: Hippotragini; Ovisovini; Alcelaphini; Antilopini and Neotragini; Bovini, Tragelaphini and Reduncini. A chi-square test showed that, in terms of the minimum number contents within these tribal groups, STS differs significantly from SE ($\chi^2_4 = 11.84$; $\chi^2_4(0.05) = 9.488$).

The tribes Alcelaphini and Antilopini are widely recognised as generally forming the bulk of any open plains-grassland fauna. For STS and SE the percentages which the added minimum numbers of these two tribes constitute of the total minimum numbers were found to be 48% and 82% respectively (Fig. 2). A chi-square test performed on these data showed that the SE assemblage has a highly significantly larger proportion of alcelaphines and antilopines ($\chi^2_1 = 7.17$; $\chi^2_1(0.01) = 6.635$) than has the STS assemblage.

Two types of distance functions were used to express affinity, in terms of minimum numbers per species, between all pairs of site units in Table 1. The first, here named FCD for 'faunal composition difference' coefficient, was arrived at as follows: if the taxa (species) are coded $i = 1, \dots, I$ and the site units $j = 1, \dots, J$ and if x_{ij} denotes the minimum number of individuals of species i at site unit j , then the FCD between site units m and n , denoted by D_{mn} , is calculated as

$$D_{mn} = \sum_{i=1}^I \left| P_{im} - P_{in} \right|$$

where $P_{ij} = \frac{x_{ij}}{x_{.j}}$, $j = 1, \dots, J$, where $x_{.j}$ denotes the sum of x_{ij}

over i . FCDs range from 0 to 2. The second distance function used was χ^2 . Each of the two functions was calculated (a) for all pairs of site units over all separate species categories 1–24; (b) for all pairs of site units over separate species categories 1–11 and 16–24, but lumping 12–15 into a single category; (c) for all pairs of site units with total minimum numbers ≥ 3 over all separate species categories 1–24; (d) for all pairs of site units with total minimum numbers ≥ 3 over separate species categories 1–11 and 16–24, but lumping 12–15 into a single category. The resulting matrices of FCDs and χ^2 values were clustered by the Weighted Pair Group Method Clustering Procedure⁸ into several dendrograms. An example is given in Fig. 3. Without exception the dendrograms showed the most fundamental split to be between STS, together with D13, D14 and D15, and the rest. A further split among the remaining site units was consistently indicated between SE together with H2, D1 and D5 on the one hand, and D16 together with D8 and D6 on the other. The placement of D2, D3 and D12, each with a total minimum number of 1, varied, but they were always separate from the STS cluster. Figure 4 shows the three faunal phases represented by site units, as suggested by these methods. It must be borne in mind that the low minimum number content of some of the site units limits the confidence that can be felt in the accuracy of their placement.

Bovoid faunal phases

A comparison between Figs 1, 3 and 4 shows an interesting correspondence between the geographic location of site units and their grouping on bovid content, with the possible exception of D5 and D8. Nonetheless there remains a greater measure of uncertainty with respect to the homogeneity of the dump assemblages than is the case with STS and SE. It is therefore useful to look first at the bovid species distribution between faunal phases A and B as represented only by STS and SE (Fig. 3). The dominant species at STS are: *Makapania broomi*, which is also among the most common species at the Makapansgat Limeworks⁹; a large new hippotragine species, which could be ancestral to *Hippotragus gigas* known throughout the Olduvai Beds and at Elandsfontein in South Africa; and a small alcelaphine which could be close to, or specifically identical to, a species from the East Rudolf *Metridiochoerus*

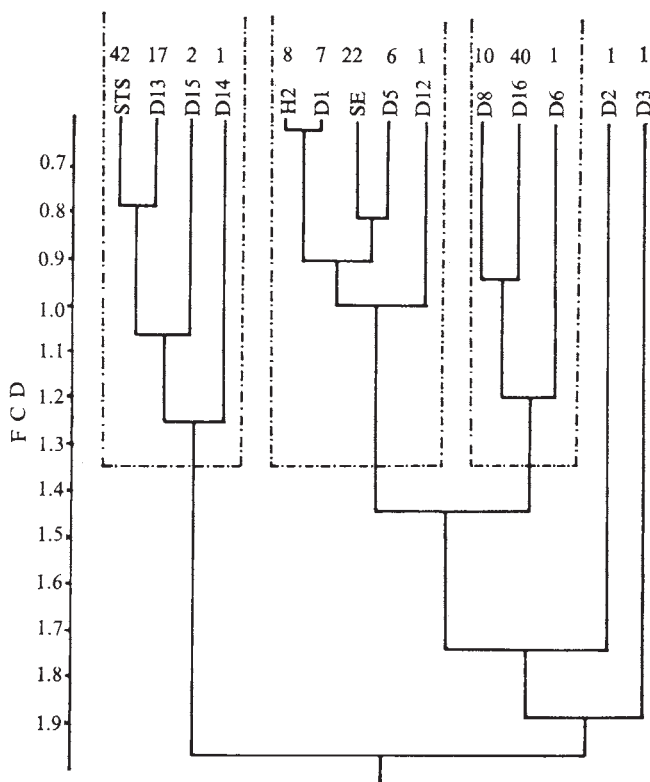


Fig. 3 Dendrogram of the relations among 14 site units based on WPGM clustering procedure⁸ using FCD values. Site units as in Fig. 1, each accompanied by its minimum number content.

andrewsi zone¹⁰. Each of the three species is represented doubtfully in the SE assemblage by a single broken specimen. At SE can be seen the first occurrence in the Sterkfontein context of the distinctive dentitions indistinguishable from the extant *Damaliscus dorcas*. *Antidorcas bondi* and *Damaliscus* sp. 2, which is likely to be *Damaliscus niro* (my unpublished work), probably also make their first appearance at SE, if the single specimen of each from STS is indeed misplaced in that assemblage as I strongly suspect for several reasons. On the whole, in view of the markedly greater affinity to Makapansgat of STS than of SE, and the implications of the χ^2 test results, the bovid assemblages of STS and SE seem to be essentially different and successive in time.

Figure 4 shows that inclusion of the dumps amplifies differences between faunal phases A and B, and adds a third phase. Faunal phase C, as represented mainly by D16 is undoubtedly distinct in its general character from phase B, and probably considerably later in time. There is here the advent at Sterkfontein of several species that are indistinguishable from recent forms. Only two phase C species are definitely extinct: *A. bondi* and the 'larger small' *Damaliscus* sp., which again in phase C very likely belongs to *D. niro* (my unpublished work). *A. bondi* and *D. niro* are generally among the few extinct bovid species at southern African Middle Stone Age sites.

It seems that at least a large part of the differences between these three bovid faunal phases, accumulated in close geographical proximity and signalling the advent of new 'indistinguishable-from-recent' species with each successive phase, is due to time. Phase A, associated with the STS australopithecine assemblage, belongs with Makapansgat in the Sterkfontein Faunal Span. Phase B, associated with the SE hominids which might include at least some *Homo*, resembles both Kromdraai A (the faunal site) and Swartkrans with respect to its high alcelaphine-antilopine content, although it is perhaps closer to the latter in terms of species present. It could belong to the Swartkrans or to the Cornelia Span. Relevant here is the simi-

larity of tool forms from both SE and Swartkrans to the developed Oldowan assemblages¹¹. Phase C could belong to the Florisbad⁷ or Florisbad-Vlakkras Faunal Span⁶ of Middle Stone Age time. In my thesis for the University of Cape Town, I am making a more detailed attempt at bovid faunal correlation

between the Sterkfontein phases and the other Krugersdorp sites. Tobias¹² has reviewed the dating of the South African australopithecine sites, linking the results of new geomorphological studies to what has so far been concluded on the basis of faunal comparisons.

	Faunal phase A	Faunal phase B	Faunal phase C	Species also present (x) or probably present (?x) at Makapans- gat Lime- works
Primary site units; min. nos. > 15 :	STS D13	SE	D16	
Secondary site units; min. nos. = 5-15:		H2 D1 D5	D8	
Tertiary site units; min. nos. = < 5 :	D14 D15	D3 D12 D2	D6	
<i>Hippotragus</i> cf. <i>equinus</i> (IR roan antelope)				
cf. <i>Megalotragus</i> sp. (E large hartebeest type)				
<i>Redunca</i> cf. <i>arundinum</i> (IR reedbuck)				?X
<i>Syncerus</i> cf. <i>acoelotus</i> (E buffalo)				?X
<i>Tragelaphus</i> sp. aff. <i>angasi</i> (E nyala)				?X
cf. <i>Gazella vanhoepeni</i> (E gazelle)				X
<i>Makapania</i> cf. <i>broomi</i> (E muskox type)				X
cf. <i>Hippotragus</i> sp. aff. <i>gigas</i> (large hippotragine)				X
<i>Oreotragus major</i> (E klipspringer)				X
<i>Antidorcas</i> cf. <i>recki</i> (E springbok type)				
<i>Antidorcas bondi</i> (E springbok type)				
<i>Damaliscus</i> cf. <i>dorcas</i> (IR blesbok)				
<i>Kobus</i> cf. <i>ellipsiprymnus</i> (IR waterbuck)				
<i>Raphicerus</i> cf. <i>campestris</i> (IR steinbok)				
<i>Antidorcas</i> cf. <i>marsupialis</i> (IR springbok)				
<i>Ourebia</i> cf. <i>ourebi</i> (IR oribi)				
<i>Pelea</i> cf. <i>capreolus</i> (IR Vaal ribbok)				
<i>Tragelaphus</i> cf. <i>scriptus</i> (IR bushbuck)				
<i>Hippotragus</i> cf. <i>niger</i> (IR sable antelope)				
<i>Tragelaphus</i> cf. <i>strepsiceros</i> (IR greater kudu)				
<i>Taurotragus</i> cf. <i>oryx</i> (IR eland)				
<i>Connochaetes taurinus</i> lineage (E and IR blue wildebeest)				?X
<i>Damaliscus</i> sp. (larger small) (E blesbok type)				
Medium-sized alcelaphines (E and IR)				?X
% constituted by min. no. of alcelaphines and antilopines of total min. no. in a faunal phase:	$\frac{25 \times 100}{62 \quad 1} = 40\%$	$\frac{37 \times 100}{43 \quad 1} = 86\%$	$\frac{32 \times 100}{51 \quad 1} = 63\%$	
% constituted by min. no. of definitely extinct individuals of total min. no. in a faunal phase:	92%	58%	23%	

Line thicknesses represent minimum number percentages per species per faunal phase as follows:

20-29%

10-19%

5-9%

1-4%

Only 1 or 2 specimens present which are suspected of belonging to a different faunal phase

Fig. 4 Suggested distribution of bovid species throughout all site units at Sterkfontein. E, extinct; IR, indistinguishable from recent

Are there differences between phases A, B and C as here constituted, other than those resulting from time? In phases A and C, rather than B, there seem to predominate forms that are identical with or close to extant species that are to a greater or lesser extent bush-loving and water-dependent, like the roan antelope, reedbuck, buffalo, nyala, sable antelope and bushbuck. This is correlated with the significantly higher percentage of alcelaphines and antilopines in phase B. Although Fig. 4 makes it clear that some degree of open grassland existed near Sterkfontein during all three time periods, the data strongly suggest that during phase B there was proportionately less bush cover than during A and C. One possible cause of such vegetational change could be a decrease in rainfall between A and B with a subsequent increase in phase C. Brain⁵ concluded, on the basis of determinations of the sand grain angularity and chert-quartz ratios of the australopithecine breccias, that a general increase in rainfall, with minor fluctuations, occurred in the Sterkfontein valley from STS times onwards. An explanation for the bovid data in Fig. 4 invoking bush cover changes as a result of rainfall changes would thus seem to stand in contradiction to Brain's findings between phases A and B, while agreeing with them between B and C. Another strong possibility is that the apparent vegetation changes are largely or entirely due to a drop in temperature between phases A and B with a subsequent increase towards C.

From this perspective, therefore, it can be said that the Sterkfontein bovid fossils show evidence of having been accumulated not only at three different times but also under varying ecological, more specifically bushcover, conditions, the latter probably resulting from temperature and/or rainfall changes.

A further difference between phases A and B must be mentioned here. Individuals in phase A have a larger average body-size than do those in phase B. Although it is not impossible that

this effect is at least to some extent correlated with the ecological differences that were deduced on the basis of species content, it leads to speculation about a possible accumulation difference: for instance, the phase A bovid remains could largely be the result of the activities of a predator, such as a sabre-tooth cat, specialised for eating large prey. Perhaps the predominant influence contributing to the phase B bovid assemblage was the advanced hominid, who in spite of his tools may have been confined to small to medium-sized prey. Detailed studies by C. K. Brain of the mode of accumulation of the australopithecine bone assemblages, and my further studies of their bovid faunas, may throw light on such questions.

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Radiometric ages of late Cainozoic basalts from northern Israel: chronostratigraphic implications

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K-Ar dating of volcanics in the Jordan rift allows an estimation of the ages of widespread stratigraphic events in the eastern Mediterranean region. The data provide a link between Pleistocene deposits of Europe and Africa suggesting, in particular, age limits to the early Acheulian and Mousterian cultures.

BASALT volcanism of late Cainozoic age is widespread in the western part of the Arabian peninsula, along a zone trending NNW from the Red Sea into Turkey. One of the most prominent occurrences of this volcanic phase is the Jebel Druze basalt province, in which lavas, covering about 48,000 km², extend south-eastwards from Lebanon to Israel, through Syria

and Transjordan, into Saudi Arabia. K-Ar ages have been determined on basalts occurring in the north-western extremity of the province and five eruptive phases, interbedded with late Cainozoic sediments of the northern Jordan Valley and the Valley of Jezre'el, are distinguished. Our data include representative samples of the chief units throughout the late Cainozoic volcanic sequence exposed in this region, with the possible exception of some of the most recent flows from the Golan Plateau (Figs 1 and 2). The present dating project thus enables radiometric ages to be allocated to many late Cainozoic sedimentary formations and stratigraphical events on the eastern seaboard of the Mediterranean.

K-Ar age determinations

The rocks investigated here have the chemical and mineralogical characteristics of alkali olivine basalts, closely similar to those described from the Jebel Druze and other Cainozoic localities

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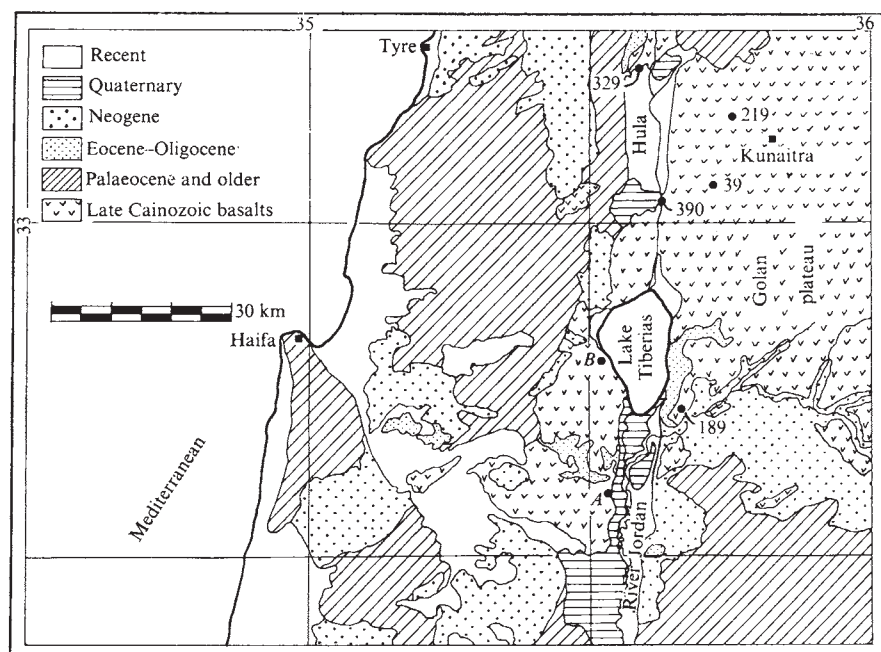


Fig. 1 Geological sketch map of northern Israel. Numbers indicate the locations of dated specimens. A, Belvoir section; B, Tiberias section.

in Transjordan^{1,2}. The analysed specimens from the northern Jordan Rift, represented in Table 1, are nonamygdaloidal, fine grained and commonly porphyritic. The phenocrysts are mostly olivine which occurs either alone or accompanied by augite and labradoritic plagioclase in various proportions. The matrix includes plagioclase in the range andesine-labradorite, augite, olivine and, in some varieties, nepheline with analcite (ref. 3, and I. Brenner, unpublished work).

Argon isotope ratios were measured statically on a Varian GD-150 mass spectrometer, equipped with a vibrating reed electrometer, using an Ar-38 enriched spike. A correction of 295.5 for the atmospheric $^{40}\text{Ar} : ^{39}\text{Ar}$ ratio was used in calculating the ages. Peak magnitudes were calculated by extrapolation

from successive scans back to the opening of the Ar-extraction line to the mass spectrometer. The decay constants adopted were

$$\lambda \beta = 4.72 \times 10^{-10} \text{ yr}^{-1}$$

and

$$\lambda e = 0.584 \times 10^{-10} \text{ yr}^{-1}$$

and the $^{40}\text{K} : \text{K}$ ratio = 1.19×10^{-2} atom %. Potassium was determined by atomic absorption on a Perkin-Elmer 303 instrument, using a bracketing procedure for calculating the results. The errors in the individual ages (Table 1) reflect analytical uncertainty only and were calculated for each analysis at the one-sigma level, following the method outlined by McDougall *et al.*⁴.

Table 1 Occurrence, locations K-Ar analytical data, and calculated ages of late-Cainozoic basalts from northern Israel

Sample no.	Rock type and field occurrence	Formation	Location (height above sea level in brackets)	Lat. N	Long. E	Geo-magnetic polarity	K (weight %)	Radiogenic Ar ($10^{-11}\text{mol/g}^{-1}$)	Radiogenic ^{40}Ar Total ^{40}Ar (%)	Calculated age (Myr)	Stratigraphic age
1B-219	Basalt flow	El Furn	Golan Plateau, El Furn (+900 m)	33°09', 35°45.5'		N	1.410	0.019	0.8	0.079 ± 0.013	Riss-Würm
1B-39	Basalt flow	Aleka	Golan Plateau, Aleka (+520 m)	33°03', 35°42.3'		N	1.192	0.012	0.5	0.064 ± 0.013	
1B-329	Basalt flow	Hasbani	Northern Jordan Valley, Qiriat Shemoa (+100 m)	33°13.5', 35°35.5'			1.056	0.014	0.6	0.073 ± 0.014	
1B-189	Basalt flow	Yarmuk	Eastern Jordan Valley, above Yarmuk River (+220 m)	32°42.5', 35°39'			0.709	0.085	4.9	0.68 ± 0.05	Late Mindel or early Mindel-Riss
1B-390	Basalt flow	Yarda	Eastern Jordan Valley, near B'not Yaakov (+100 m)	33°01', 35°58'		N	0.938	0.107	6.9	0.64 ± 0.12	
1B-48	Basalt flow, near top	Cover Basalt	Jordan Valley, Tiberias section (+180 m)	32°47.1', 35°31.6'		R	1.222	0.33	15.7	1.7 ± 0.1	Preglacial Pleistocene
1B-274	Basalt flow, near base	Cover Basalt	Jordan Valley, Belvoir section (+250 m)	32°35.5', 35°32.3'		R	0.687	0.26	8.8	2.0 ± 0.1	
1B-82	Basalt flow	Intermediate Basalt	Jordan Valley, Belvoir section Single flow-samples about 100 m apart (+200 m)	32°35.5', 35°32.3'	N		1.245	1.06	14.7	4.7 ± 0.2	Middle Tertiary
1B-185	Basalt flow						0.668	0.60	7.1	5.0 ± 0.3	
1B-187	Basalt flow						0.886	0.74	2.2	4.7 ± 0.5	
1B-180	Basalt flow, uppermost	Lower Basalt	Jordan Valley Belvoir section (+90 m)	32°35.5', 35°32.3'		N	1.09	2.45	17.8	12.5 ± 0.5	Middle Miocene
1B-172	Basalt flow	Lower Basalt	Jordan Valley, Belvoir section (-150 m)	32°35.5', 35°32.3'		N	1.172	3.06	38.5	14.6 ± 0.4	
1B-86	Basalt flow, lowermost	Lower Basalt	Jordan Valley, Belvoir section (-200 m)	32°35.4', 35°32.3'		N	1.259	2.90	67.6	13.0 ± 0.3	
1B-84	Basalt flow, lowermost	Lower Basalt	Jordan Valley, Tiberias section (-90 m)	32°47.1', 35°31.6'		N	0.826	1.78	24.1	12.2 ± 0.4	

In spite of the large errors shown for the Quaternary basalts, which mainly reflect the high level of atmospheric argon contamination, the calculated ages are clustered closely into groups differing by an order of magnitude, and are in excellent agreement with available stratigraphic evidence. Thus, on the present data, the pattern of the post-Middle Pliocene volcanism in the northern Jordan Valley region seems to have comprised several intense but discrete episodes.

Stratigraphy

The earliest manifestations of Cainozoic volcanism in the Jebel Druze igneous province are provided by several basalt horizons of Eocene and Miocene age penetrated by boreholes in Transjordan¹. Near Tripoli and Damascus, Neogene volcanism began in the Middle Miocene⁵ and on the northern side of Wadi Sirhan, in north-western Saudi Arabia, several extensive basalt flows have yielded an average K-Ar age of 12.3 Myr (ref. 6). In northern Israel some 500 m of Lower Basalt flows of Middle Miocene age (Table 1) interdigitate with fluvial sediments of the Herod Formation⁷. The latter correlates with the Hazeva Formation of the Negev, which interfingers in the Be'er Sheva area with Middle Miocene marine sediments of the Ziqliq Formation^{8,9,25}. The Herod and Hazeva Formations are regarded as representing the maximum extent of the Middle Miocene marine transgression²⁵. Sediments from the Barada Valley near Damascus, which resemble the Herod Formation in age and lithology, and include interbedded basalt flows, have been described by Dubertret⁶. The dated flows of Lower Basalt occur on the western rift scarp of the northern Jordan Valley, at the Belvoir and Tiberias sections (Fig. 1) and are 12.2 to 14.6 Myr old. The flows may be regarded as essentially coeval, with a mean age of 13.1 Myr.

The Herod Formation is unconformably overlain by the Bira and Gesher Formations, within which several flows of the Intermediate Basalt are intercalated. The latter has been described by Schulman⁷ for the central Jordan Valley, and by Horowitz¹⁰ and Fleischer (Subsurface geology of the Hula, Geol. Surv. Ist. Rep., 1968. Unpublished) for the Hula Valley and Korazim Block, respectively. The Bira Formation, mainly lagoonal chalks and marls, interfingers westward in the Yizre'el Valley with marine sediments bearing a typical Pliocene fauna^{8,11,12}. Bira sediments of the central Jordan Valley, where the dated flow is located, are interpreted as representing the Tabianian stage of the Mediterranean Pliocene²⁵, as recently defined¹³. At Belvoir a single flow was sampled at three locations about 100 m apart in order to test the reproducibility of our measurements. Individually calculated ages of the Intermediate Basalt agree to within about 3% of their mean of 4.8 Myr.

Volcanism recommenced throughout the Jebel Druze province with the formation of plateau basalts which inundated wide areas in north-western Transjordan, south-western Syria, Lebanon, and north-eastern Israel. In the last of these areas the Cover Basalt commonly attains thicknesses of 100 to 150 m and most sections show at least 10 flows, each 10 to 20 m thick⁷. The Cover Basalt is separated by an erosional unconformity from underlying Pliocene formations and is itself overlain by Guenzian sediments of the Jordan Valley^{10,25}. No sediments interfinger with the Cover Basalt, but it has been stratigraphically correlated with the preglacial Pleistocene formations of the Coastal Plain, Judea and the Negev in Israel²⁵. Ages determined on flows from near the top of the Belvoir and Tiberias sections agree closely and have an average age of 1.8 Myr.

The results given here enable us to distinguish two age groups in the post-Cover Basalt volcanics: one with an average age of 0.66 Myr, the other much younger at near 72×10^3 yr. Carbon-14 dates from bones buried within the youngest lavas on the Syrian Jebel Druze indicate an age of 4.5×10^3 yr (ref. 14). In the Hula Valley, Quaternary basalts occur in tilted blocks, interbedded with Pleistocene deposits and, on the northern border, as flat-lying sheets which can be traced to the Golan

Plateau. The Yorda Basalt^{11,15} flowed over the Korazim-Gadot block, south of the Hula Valley, filling erosional features in the Guenzian Gadot Formation and in the Mindelian Mishmar Hayarden Formation. The faulted and tilted Yorda basalt is unconformably overlain by the Russian Benot Ya'akov Formation. Its stratigraphical age should, therefore, correspond to the late Mindel or the early stage of the Mindel-Riss interpluvial. The Yorda Basalt has been correlated by Horowitz¹⁰ with the Yarmuk Basalt¹¹ of the central Jordan Valley. Radiometric ages of these basalts are 0.64 and 0.68 Myr respectively.

The youngest extrusive episode studied by us is represented by two flows from the Golan Plateau and one from the northern Hula Valley, all of which are coeval within the analytical error, with a mean age of 72×10^3 Myr and normal magnetic polarity (D. Mor, personal communication). The Hasbani Basalt covers the upper Hula Valley¹⁵, extending northwards into Lebanon and eastwards to its probable source in the Golan. This basalt, sampled as a surface flow near Qiriat Shemona (1B-329), has been traced to the Banias waterfall¹⁰ where it separates two travertine layers. The underlying Kefar

Specimen Number	K Ar ages ($\times 10^6$ yr)	Chrono-stratigraphy	Hula Basin	Central Jordan Valley
		Holocene	Majaha Formation	Tabgha Formation
		Würm	Dan Travertine Ashmura Formation	Lisan Formation
39 329 219	0.064 0.073 0.079	R-W	Hasbani Basalt and Hulata Formation	Roqqad (Naharayim) Basalt
		Riss	Benot Ya'akov Formation and Kefar Yuval Travertine	Naharayim Formation
		M-R	Ayelet Hashahar Formation	
390 189	0.64 0.68		Yorda Basalt	Yarmuk Basalt
		Mindel	Mishmar Hayarden Formation	Ubeidiya Formation
		G-M	Palaeosol	
48 274	1.7 2.0	Günz	Gadot-Hazor Fm.	Erk el. Ahmar Fm.
		Preglacial Pleistocene	Cover Basalt	
		Pliocene	Tanur Conglomerate	Fejjas Tuff
82 185 187	4.8	Tabianian	Tel Hai Limestone	Geshel Fm. I. Basalt Um Sabune Cgl.
		Miocene		
84 180 86 172	12.2 12.5 13.0 14.6			Lower Basalt Herod Fm.

Fig. 2 Upper Cainozoic sections for northern Israel, showing stratigraphical positions of dated basalts. (I. Basalt = Intermediate Basalt).

Yuval Travertine contains late Acheulian implements and corresponds stratigraphically to the Rissian Benot Ya'akov Formation of the Hula Valley¹⁰. The Dan Travertine overlies the Hasbani Basalt and can be correlated with the Würmian Ashmura Formation of the Hula Valley which contains implements of Mousterian to Epipalaeolithic age, and with the Roqqad (or Naharayim) Basalt¹⁶ of the central Jordan Valley with which it is stratigraphically analogous¹⁰. The Hasbani Basalt thus constitutes a precise marker for the calibration of the Riss-Würm interpluvial. The age of 73×10^3 yr indicated for this phase agrees with estimates of sedimentation rates and radiocarbon age determinations of the Würmian sequence in Israel^{8,10,17}. The sampled flows from the Golan Plateau do not interdigitate with any sediments and are not subject to direct stratigraphic control, but their radiometric ages clearly show that they correlate with the Hasbani Basalt.

Discussion

The maximum development of the Miocene marine transgression in the Mediterranean region, allocated a Middle Miocene age by Gignoux¹⁸, has been shown by our dating of the Lower Basalt to have occurred between 12.2 and 14.6 Myr ago, which is in good agreement with evidence from other regions¹⁹. The Tabianian marine transgression, represented in Israel by sediments of the Pliocene Bira Formation, which are about 4.8 Myr old, is thought to have been very rapid, and to have reached its maximum extent within about 0.5 to 0.75 Myr (ref. 20).

The age of the Cover Basalt is relevant to the dating of the preglacial and the beginning of the Pleistocene glacial stages. The dated specimens were collected from the upper flows of this volcanic sequence and represent the end of the preglacial Pleistocene, about 1.7–2.0 Myr ago. The reversed magnetic polarity of the upper section of the Cover Basalt, and the consistently normal polarity of its lowermost flows^{21,22}, strongly suggests that its eruption straddled the transition from the Gauss Normal to the Matuyama Reversed epochs, estimated at 2.4 Myr (ref. 23). The beginning of the preglacial Pleistocene therefore predates the polarity transition and we propose, provisionally, to allocate it an age in the range 2.6 to 3.0 Myr—a date which also corresponds to the close of the Pliocene.

The radiometric dating of the Yorda and Yarmuk Basalts, stratigraphically of late or post-Mindel age, indicates a minimum age of 0.66 Myr for the Mindel. This age limit provides an important link between the early Acheulian cultures of Europe and Africa and has been discussed in detail²⁴. Radiometric dates from Pleistocene deposits in Africa are fairly numerous, but in Europe there is almost no dateable Pleistocene material. Furthermore, climatic correlation between the European and African Pleistocene is poor, whereas the correlation between the European and Israeli Pleistocene seems well established¹⁷. Dating of the Israeli Pleistocene thus provides a sound basis for Pleistocene geochronology in Europe.

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Vegetation classification by reference to strategies

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It is suggested that there are three major determinants of vegetation—competition, stress and disturbance—and that each has invoked a distinct strategy on the part of the flowering plant. A method is described whereby it is possible to distinguish types of herbaceous vegetation by reference to the relative importance of the three strategies in the genotypes of the component species.

THE electronic computer has been a mixed blessing to vegetation classification. On the one hand, it has facilitated the development of methods of numerical and multivariate analysis. On the other, it has contributed to the decline in confidence in

established methods but has yet to replace them with a new *lingua franca*. The resultant confusion in the field of vegetation classification is unfortunate in that it coincides with an unprecedented demand for standardised botanical information which can be readily interpreted and assimilated into plans to reclaim or manage the landscape.

This is not to argue for a return to the older methods of phytosociology which apart from their subjectivity are often difficult to apply in a world landscape experiencing increasingly diverse and disruptive interference by man. The current requirement is for methods of classification which can include recent or unstable vegetation, avoid unnecessary abstraction and provide data intelligible to nonspecialists. An approach to the classification of herbaceous vegetation has been made with these considerations in mind.

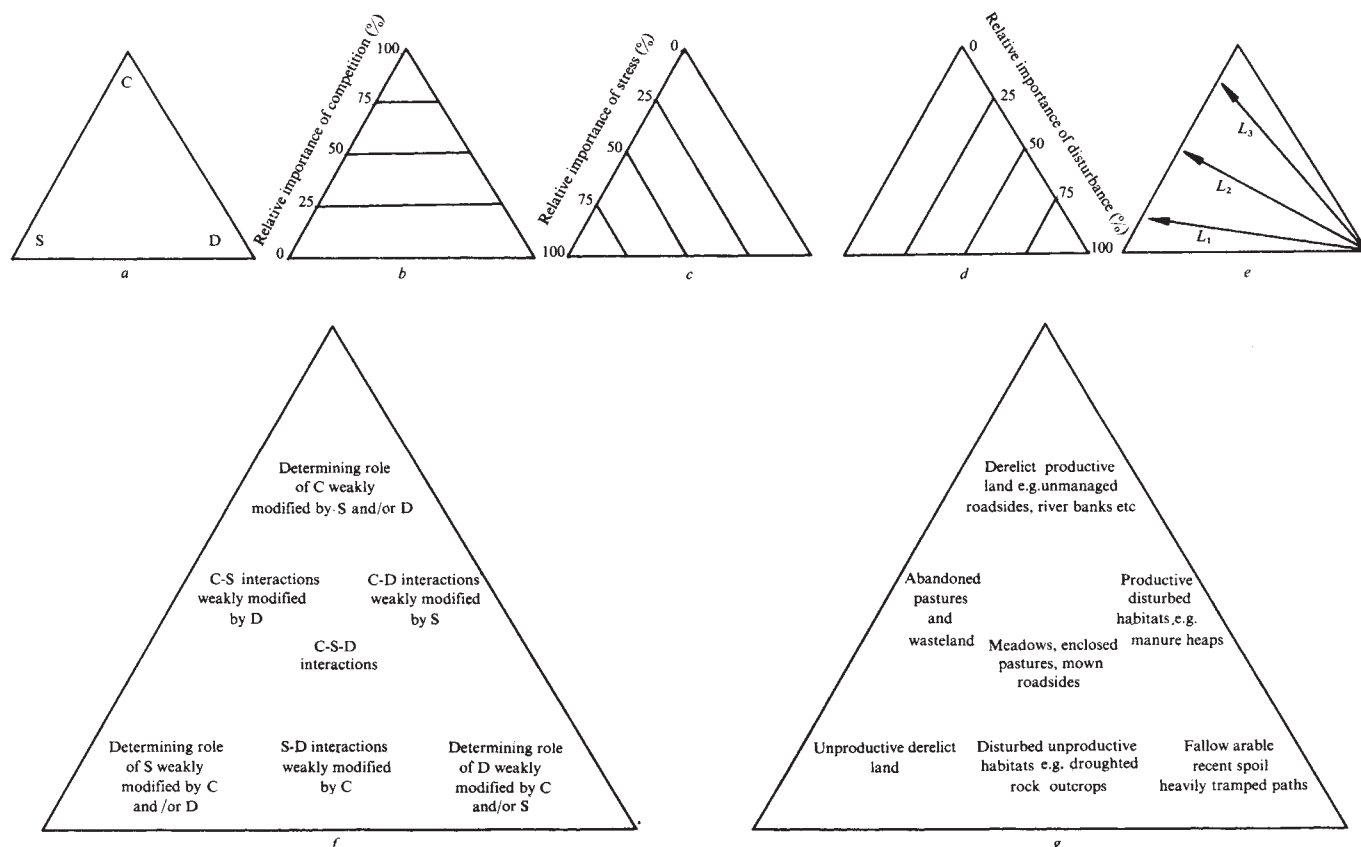


Fig. 1 A triangular model of herbaceous vegetation. *a*, Identification of the corners at which competition (C), stress (S) and disturbance (D) are exclusive determinants. *b-d*, Contours in percentage contribution of competition, stress and disturbance, respectively. *e*, Course of vegetation succession. The arrows correspond to lines of succession of low (L_1), moderate (L_2) and high (L_3) productivity. *f*, Interaction of competition, stress and disturbance. *g*, Location of selected habitat types.

Triangular ordination

The method depends upon the assertion that, basically, there are three determinants of herbaceous vegetation—competition, stress and disturbance—and that each has invoked a distinct strategy on the part of the flowering plant. The possibility arises therefore that an ordination of vegetation types can be based on measurements of the relative importance of the three strategies among the component species. More specifically, it is suggested that the spectrum of herbaceous vegetation types may be accommodated in a model (Fig. 1*a*) consisting of an equilateral triangle in the corners of which the relative importance of competition (C), stress (S) and disturbance (D) reach their respective maxima. In Fig. 1*b-d*, the gradients in relative importance of each of the three determinants are indicated by means of contours and in Fig. 1*f* the interactions between competition, stress and disturbance are summarised. Figure 1*g* illustrates the predicted location of selected habitat types within the triangle. The triangular model also appears to incorporate some of the dynamic features of herbaceous vegetation. The course of succession (Fig. 1*e*) is from the right-hand corner of the triangle towards the opposite side which constitutes the interface with woody vegetation. Increase in the angle of elevation of the line of succession (L_1 – L_3) above the horizontal is associated with a progressive increase in productivity. Species density (number of species per unit area) would be expected to show a progressive decline towards the apex as a result of competitive exclusion.

To derive from this model a practical method of ordination, the minimum requirement is to find measurable attributes of the flowering plant which vary in accordance with any two of the three sets of contours in Fig. 1*b-d*. To explore the possible means by which this requirement may be fulfilled it is necessary to consider the essential nature of competition, stress and disturbance and the strategies which they seem to have invoked.

Competition, stress and disturbance

Competition may be defined as the attempt by neighbouring plants to utilise the same units of light, water, mineral nutrients or space¹. By definition, therefore, competition exerts its maximum impact as a determinant of vegetation in circumstances where the competition is resolved perhaps even to the extent that the habitat is occupied by one species, possibly one individual plant. Stress and disturbance together comprise those phenomena which prevent the resolution of competition. At moderate intensities this intervention has the effect of creating spatial or temporal niches; at their most severe both stress and disturbance may so suppress plant development that individual plants scarcely impinge on each other and competition is occluded. The difference between stress and disturbance lies in the fact that whilst both inhibit the development of a large standing crop the former does so by restricting primary production, the latter by damage to the vegetation. Whereas stress is usually imposed by the physical environment (shortages of light, water, mineral nutrients, suboptimal temperatures, soil and toxins), disturbance arises from the activities of grazing animals, pathogens, man (trampling, mowing and ploughing) and from physical phenomena such as soil erosion. The same environmental factor, drought for example, may cause both stress and disturbance. In certain situations it is difficult to distinguish between competition and stress. In particular, problems of definition arise where herbaceous vegetation derives shade or phytotoxins from a remote tree canopy.

Three strategies

Many plant attributes have been implicated in adaptations to particular forms of competition, stress or disturbance. Here attention is focused on general characteristics of the competitive, stress-tolerant and ruderal strategies.

The competitive strategy. From both field and laboratory investigations of competition²⁻¹⁵, it seems that a number of plant attributes are conducive to the efficient capture and utilisation of light, water, mineral nutrients and space. These include an elevated leaf canopy, the capacity for extensive lateral spread both above and below ground and the tendency to accumulate a thick layer of litter on the ground surface, all characteristics which are especially prominent in herbaceous species such as *Pteridium aquilinum* and *Epilobium hirsutum*, which frequently occupy extensive areas of vegetation to the virtual exclusion of

other species. The ability of these three attributes to distinguish the competitive strategy is suggested by their very low incidence in environments subjected either to severe stress or to continuous disturbance¹⁶.

The stress tolerant strategy. In addition to small stature, a general characteristic of herbaceous plants in environments experiencing continuous and severe stress is a low potential relative growth rate¹⁷. This generalisation appears to hold for a wide variety of stresses including those associated with nutrient deficiencies on basic and acidic soils¹⁸⁻²³, shading^{24,25} and

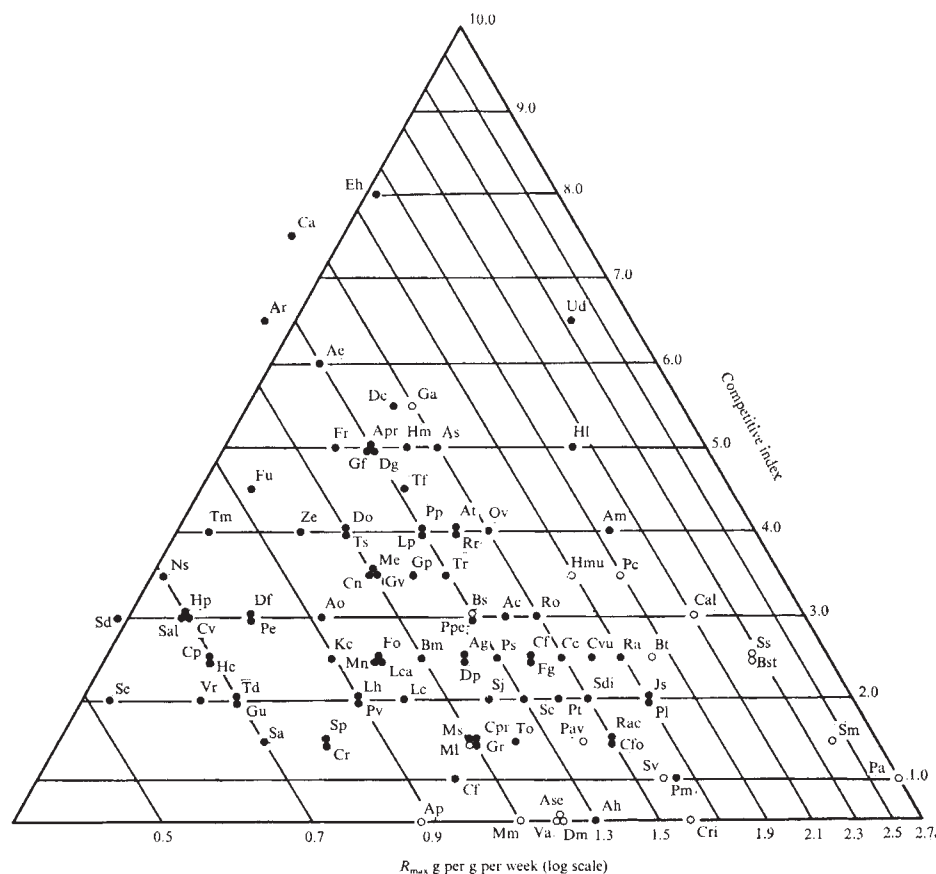


Fig. 2 A triangular ordination of herbaceous species. ○, Annuals; ●, perennials (including biennials). The competitive index (CI) was calculated from the formula $CI = (a+b+c)/2$ where *a*, estimated maximum height of leaf canopy (1, < 12 cm; 2, 12–25 cm; 3, 25–37 cm; 4, 37–50 cm; 5, 50–62 cm; 6, 62–75 cm; 7, 75–87 cm; 8, 87–100 cm; 9, 100–112 cm; 10, > 112 cm); *b*, lateral spread (0, small therophytes; 1, robust therophytes; 2, perennials with compact unbranched rhizome or forming small (< 10 cm diameter) tussock; 3, perennials with rhizomatous system or tussock attaining diameter 10–25 cm; 4, perennials attaining diameter 26–100 cm; 5, perennials attaining diameter > 100 cm); *c*, estimated maximum accumulation of persistent litter (0, none; 1, thin, discontinuous cover; 2, thin, continuous cover; 3, up to 1 cm depth; 4, up to 5 cm depth; 5, > 5 cm depth).

Key to species: The value in brackets refers to the 95% confidence limit for R_{max} .

Ac, *Agrostis canina*, ssp. *canina* (± 0.18); Ae, *Arrhenatherum elatius* (± 0.16); Ag, *Alopecurus geniculatus* (± 0.14); Ah, *Arabis hirsuta* (± 0.28); Am, *Achillea millefolium* (± 0.57); Ao, *Anthoxanthum odoratum* (± 0.17); Ap, *Aira praecox* (± 0.14); Apr, *Alopecurus pratensis* (± 0.22); Ar, *Agropyron repens* (± 0.11); As, *Agrostis stolonifera* (± 0.22); Ase, *Arenaria serpyllifolia* (± 0.30); At, *Agrostis tenuis* (± 0.25); Bm, *Briza media* (± 0.14); Bs, *Brachypodium sylvaticum* (± 0.28); Bst, *Bromus sterilis* (± 0.25); Bt, *Bidens tripartita* (± 0.28); Ca, *Chamaenerion angustifolium* (± 0.17); Cal, *Chenopodium album* (± 0.73); Cc, *Cynosurus cristatus* (± 0.13); Cf, *Carex flacca* (± 0.24); Cfl, *Cardamine flexuosa* (± 0.18); Cfo, *Cerastium fontanum* (± 0.19); Cn, *Centaurea nigra* (± 0.15); Cp, *Carex panicea* (± 0.25); Cpr, *Cardamine pratensis* (± 0.18); Cr, *Campanula rotundifolia* (± 0.36); Cri, *Catapodium rigidum* (± 0.38); Cv, *Clinopodium vulgare* (± 0.09); Cvu, *Cirsium vulgare* (± 0.56); Dc, *Deschampsia cespitosa* (± 0.12); Df, *Deschampsia flexuosa* (± 0.18); Dg, *Dactylis*

glomerata (± 0.14); Dm, *Draba muralis* (± 0.15); Do, *Dryas octopetala* (± 0.47); Dp, *Digitalis purpurea* (± 0.34); Eh, *Epilobium hirsutum* (± 0.13); Fg, *Festuca gigantea* (± 0.41); Fo, *Festuca ovina* (± 0.14); Fr, *Festuca rubra* (± 0.16); Fu, *Filipendula ulmaria* (± 0.26); Ga, *Galium aparine* (± 0.21); Gf, *Glyceria fluitans* (± 0.19); Gp, *Galium palustre* (± 0.15); Gr, *Geranium robertianum* (± 0.13); Gu, *Geum urbanum* (± 0.38); Gv, *Galium verum* (± 0.30); Hc, *Helianthemum chamaecistus* (± 0.24); Hl, *Holcus lanatus* (± 0.28); Hm, *Holcus mollis* (± 0.16); Hmu, *Hordeum murinum* (± 0.35); Hp, *Helictotrichon pratense* (± 0.09); Js, *Juncus squarrosus* (± 0.17); Kc, *Koeleria cristata* (± 0.11); Lc, *Lotus corniculatus* (± 0.14); Lca, *Luzula campestris* (± 0.13); Lh, *Leontodon hispidus* (± 0.14); Lp, *Lolium perenne* (± 0.13); Me, *Milium effusum* (± 0.16); Ml, *Medicago lupulina* (± 0.13); Mm, *Matricaria matricarioides* (± 0.22); Mn, *Melica nutans* (± 0.11); Ms, *Myosotis sylvatica* (± 0.12); Ns, *Nardus stricta* (± 0.16); Ov, *Origanum vulgare* (± 0.22); Pa, *Poa annua* (± 0.42); Pav, *Polygonum aviculare* (± 0.12); Pc, *Polygonum convolvulus* (± 0.56); Pe, *Potentilla erecta* (± 0.23); Pl, *Plantago lanceolata* (± 0.13); Pm, *Plantago major* (± 0.19); Pp, *Poa pratensis* (± 0.14); Ppe, *Polygonum persicaria* (± 0.13); Pp, *Polygonum sanguisorba* (± 0.38); Pt, *Poa trivialis* (± 0.25); Pv, *Prunella vulgaris* (± 0.14); Ra, *Rumex acetosa* (± 0.33); Rac, *Rumex acetosella* (± 0.17); Ro, *Rumex obtusifolius* (± 0.29); Rr, *Ranunculus repens* (± 0.35); Sa, *Sedum acre* (± 0.17); Sal, *Sesleria albicans* (± 0.11); Sc, *Scabiosa columbaria* (± 0.16); Sd, *Sieglingia decumbens* (± 0.14); Sdi, *Silene dioica* (± 0.17); Sj, *Senecio jacobaea* (± 0.13); Sm, *Stellaria media* (± 0.23); Sp, *Succisa pratensis* (± 0.15); Ss, *Senecio squalidus* (± 0.17); Sv, *Senecio vulgaris* (± 0.36); Td, *Thymus drucei* (± 0.20); Tf, *Tussilago farfara* (± 0.17); Tm, *Trifolium medium* (± 0.08); To, *Taraxacum officinalis* (± 0.12); Tr, *Trifolium repens* (± 0.16); Ts, *Teucrium scorodonia* (± 0.21); Ud, *Urtica dioica* (± 0.13); Va, *Veronica arvensis* (± 0.28); Vr, *Viola riviniana* (± 0.16); Ze, *Zerna erecta* (± 0.08).

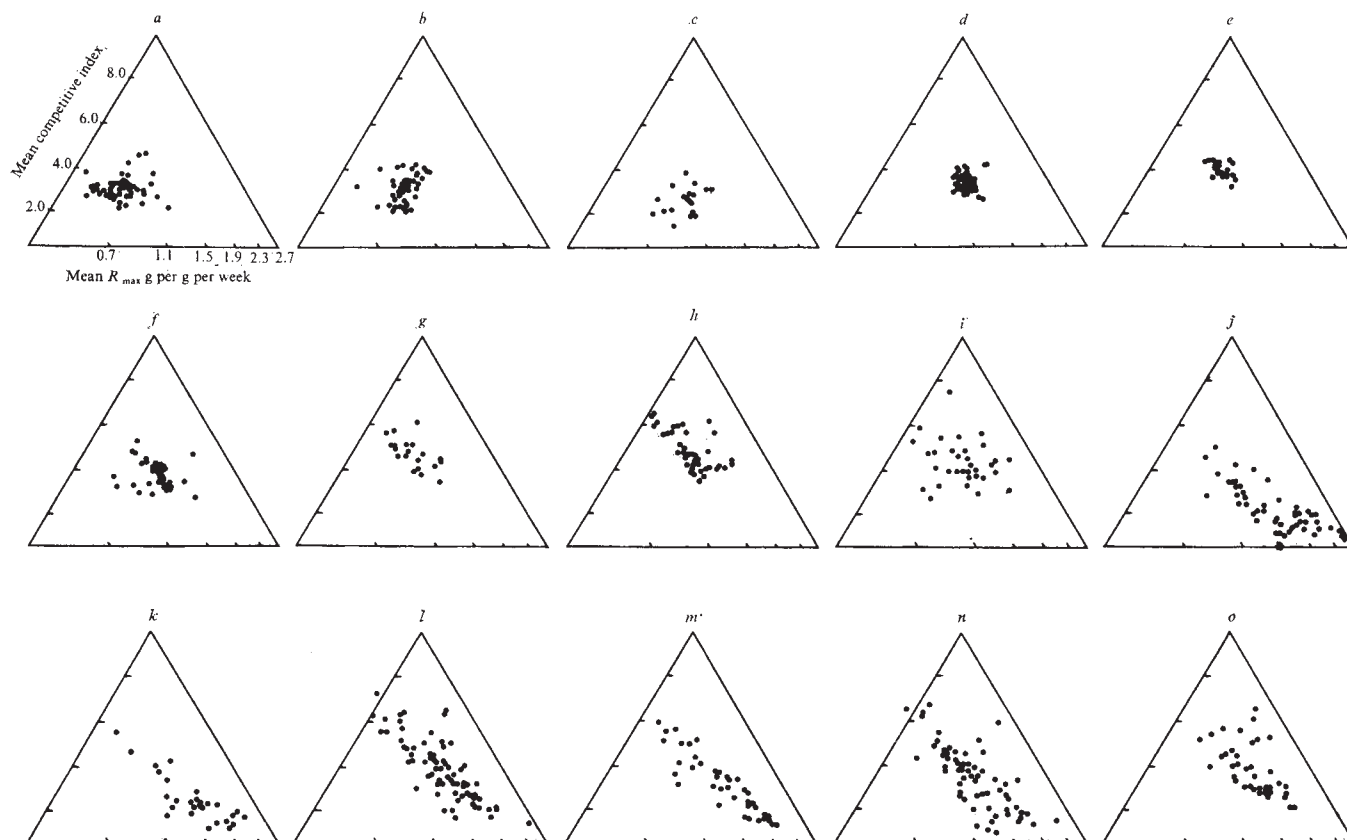


Fig. 3 Triangular ordinations of m^2 samples of herbaceous vegetation from fifteen habitats. Axes are mean competitive index and mean R_{max} each derived as in Fig. 2 and weighted according to the relative frequency of the species in the sample. *a*, Unenclosed sheep pastures on acidic strata; *b*, Unenclosed sheep pastures on limestone; *c*, limestone outcrops; *d*, meadows; *e*, road verges, mown frequently; *f*, enclosed pastures; *g*, road verges, mown infrequently; *h*, hedge bottoms; *i*, derelict banks of rivers, ponds and ditches; *j*, paths; *k*, fallow arable; *l*, heaps of mineral soil (such as building sites); *m*, demolition sites (brick and mortar rubble); *n*, cinders (tips and railway ballast); *o*, manure heaps and sewage sludge.

desiccation²³. In marked contrast, both the competitive and the ruderal strategies are associated with high potential relative growth rates^{23, 26, 27}.

The ruderal strategy. The majority of herbaceous species in highly disturbed habitats are annuals or short-lived perennials. A result of disturbance is the release of space and relief from stress and competition. It is not surprising, therefore, to find that a characteristic of many ruderals is the capacity for rapid seedling establishment and growth²³. A related feature is the tendency for a high proportion of the photosynthate to be directed into seeds and, under conditions of stress, for seed production to be maintained at the expense of vegetative growth^{28, 29}.

Ordination of species

To test the practical value of ordination by strategy, tests were carried out using data from an unpublished survey of the vegetation of the Sheffield region. As a first step, a triangular ordination was carried out on 100 herbaceous plants prominent in the data. One axis in the ordination was designed to correspond to the relative importance of the competitive strategy and was a numerical index based upon estimates of the maxima in height of canopy, lateral spread and litter accumulation derived from the field observations of Dr J. G. Hodgson and myself. The results of numerous investigations (for example refs 5–8, 11 and 13) suggest that in a majority of competitive species height of canopy is of greatest importance. For the purposes of this investigation, therefore, the maximum possible score for height of canopy was arranged to be twice that allowed for either lateral spread or litter accumulation (legend of Fig. 2). The competitive index used here differs from a predecessor¹⁶ both in the introduction of a weighting system and in the fact that relative growth rate is not incorporated. The second axis,

on a log scale, referred to stress tolerance and was R_{max} , the maximum relative growth rate of the species recorded during the period 2–5 weeks after germination in a standardised, productive growth-room environment²³. The values of R_{max} should be regarded as first estimates and may be subject to revision as data become available for other seed sources and conditions of growth.

In view of the provisional nature of the data, it is reassuring to find that the majority of the species fall within the triangle (Fig. 2) and, with certain exceptions, for example, *Origanum vulgare*, *Scabiosa columbaria* and *Tussilago farfara*, lie in close proximity to species with which they have strong ecological affinities. The left-hand corner is occupied by species tolerant of desiccation, for example *Sedum acre*, or shade, for example *Sanicula europaea*, or frequent in nutrient-deficient habitats whether acidic (*Deschampsia flexuosa*, *Nardus stricta*), calcareous (*Helictotrichon pratense*, *Sesleria albicans*) or associated with a wider range in soil pH (*Sieglingia decumbens*, *Viola riviniana*). Annual plants are concentrated in the 'ruderal corner' and show a significant ($P < 0.001$) and anticipated³⁰ rise in seed weight with increasing competitive index. Species of productive, derelict habitats occur towards the apex of the triangle. The unique position in the ordination of *Urtica dioica* is due to the unusually rapid growth rate of the species and is consistent with the sensitivity of the species to mineral-nutritional stress^{21, 23, 31}. The location of the winter annuals *Aira praecox*, *Veronica arvensis*, *Arenaria serpyllifolia* and *Draba muralis* coincides with that predicted for disturbed, unproductive habitats (Fig. 1g) and is quite distinct from that of the perennial plants such as *Festuca ovina* and *Koeleria cristata*, with which the annuals occur on limestone outcrops. This suggests that in such habitats the patches of bare soil occupied by the annuals constitute a distinct spatial and temporal niche.

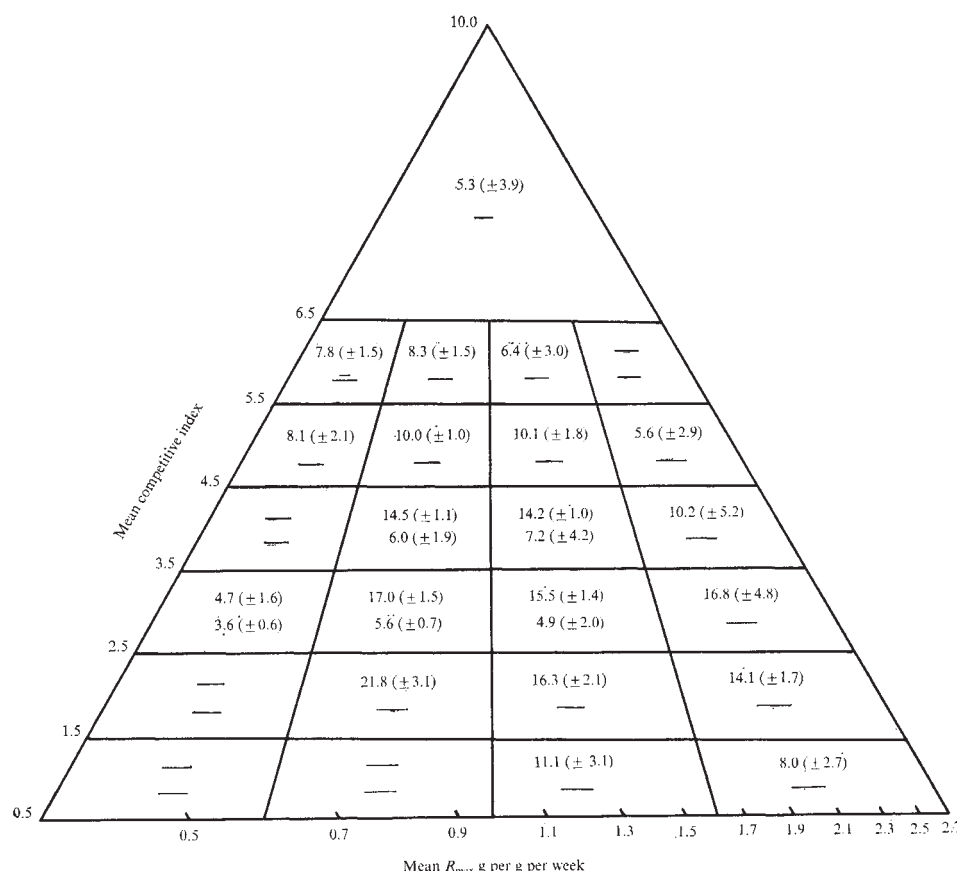


Fig. 4 Pattern of species density (number of species m⁻²) obtained by ordination of 925 samples of herbaceous vegetation from a wide range of habitats. Axes are the same as in Fig. 3. In each cell the uppermost values are the mean and 95% confidence limit for samples from sites with a surface soil pH > 4.0. The lower values refer to samples of pH < 4.1.

* Cells containing less than five samples.

Ordination of vegetation samples

The second step in evaluating the approach was to ordinate vegetation samples from a wide variety of habitats. The axes were based on mean values for individual quadrats of both the competitive index and R_{max} , with R_{max} again plotted on a logarithmic scale. In the case of the competitive index, the values for each species which contributed towards the mean for the quadrat were weighted according to the relative importance of the species in the m² sample. An identical system of weighting was applied in the calculation of mean R_{max} for the quadrat although here data were necessarily restricted to those species for which growth analyses had been carried out (> 50% and including the more frequent species in the majority of samples). A selection of the ordinations is presented in Fig. 3. The various types of vegetation occupy positions in quite close agreement with those predicted in Fig. 1g. The figures illustrate the tendency of samples from stressed habitats (a, b, c), disturbed environments (j, k) and semiderelict but productive sites (g, h, i) to extend into respective corners of the triangle. Vegetation types experiencing a moderate intensity of orderly disturbance (d, e, f) tend to occupy compact areas in the centre of the diagram. By contrast, samples from spoiled land (l-o) show an attenuated distribution which seems to represent the course of vegetation succession in these new habitats (compare Fig. 1e).

Figure 4 illustrates the pattern of species density which was obtained by pooling data from 25 ordinations (925 samples). When 103 samples from extremely acidic soils (surface soil pH < 4.1) are discounted the predicted decline in species density, towards the apex of the triangle, is apparent. The fall in species density in the lowest rank of cells is probably related to the scarcity of species adapted to extremes of stress and disturbance¹⁶.

Future developments

The consistent patterns evident in Fig. 3 are encouraging, particularly in view of the fact that the samples in each ordination have been drawn from localities scattered over a wide geo-

graphical area. However, there is obvious scope for refinement of the method. There is a need for more accurate estimations of R_{max} and for an assessment of the extent to which this attribute and others used in the ordination is subject to intraspecific variation. It is also necessary to evaluate alternative criteria with respect to both the competitive and the stress-resistant strategies. In addition, the possibility of a 'ruderal axis', perhaps related to 'reproductive effort'³² remains to be examined.

None of the species in Fig. 2 occurs within the two areas of the triangle corresponding to extreme competition or severe stress. This may be due to deficiencies in the axes or in the data. An alternative explanation is that it is necessary to explore beyond a temperate herbaceous flora in order to find the necessary conjunctions of R_{max} and competitive index.

The value of this triangular ordination to vegetation management lies at present in the ability to detect the intensity of competition and stress and to predict the intensity of disturbance at particular sites. If the approach is to be put to maximum use, however, it must be complemented by techniques which identify particular forms of competition, stress and disturbance.

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Computer-aided three-dimensional reconstruction and quantitative analysis of cells from serial electron microscopic montages of foetal monkey brain

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Computer-aided reconstruction of immature brain cells with highly irregular shapes from serial electron microscopic montages gives three-dimensional images and quantitative data on surface areas and volumes, providing new classes of data on cell behaviour during development.

KNOWLEDGE of structural relationships between cells in the central nervous system has reached a level of precision that requires efficient high resolution methods of quantitative three dimensional analysis. The classic Golgi method^{1,2} provides a blackened image of the complete silhouette of individual cells against an almost colourless background, and steps have been taken to obtain quantitative cytological data with computer aid³. But this method lacks adequate resolution and usually does not facilitate visualisation of relationships between contiguous cells. The transmission electron microscope provides appropriate resolution and facilitates visualisation of the profiles of cell bodies and processes, and their relationships with other cells in a given field of view. Preparation of several hundred consecutive serial sections is now feasible and computer-aided reconstruction methods have been applied to electron microscopic analysis of the organisation of invertebrate neural tissue (refs 4 and 5 and personal communication from S. Brenner). We describe here the application of a quantitative computer-aided method to the analysis of cell shapes,

volumes and surface areas as visualised in electron micrographs of a sector of foetal monkey cerebrum.

A 58-d embryo was removed from an anaesthetised *Macacus rhesus* monkey by hysterotomy and perfused through the circulatory system with a mixture of 1% formaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer⁶. Blocks of tissue 1×1×0.5 mm were postfixed in OsO₄, stained with uranyl acetate, dehydrated and embedded in Maraglas. Precisely oriented transverse sections 1 µm thick were cut from a sector of cerebral wall approximately at the border of the occipital and temporal lobes (Fig. 1a). The block was re-mounted and trimmed to a rhomboid shape, yielding a final block face that extended from the ventricular zone to the base of the cortical plate (Fig. 1b) and included a sector of sub-ventricular zone about 100 µm thick and intermediate zone 300 µm thick (neuroembryological terminology recommended by Boulder Committee⁷). A set of 170 consecutive serial sections, each about 80 nm thick, was mounted on Formvar film on one-hole (1×2 mm) grids and stained with uranyl acetate and lead citrate. From every third section, 16–20 overlapping fields were photographed at ×2,800 with a Hitachi electron microscope and montages were prepared of prints at a final magnification of ×8,400. Selected cells and their nuclei were outlined on transparent acetate sheets, and minor adjustments in alignments were made by reference to nearby transversely cut cell processes and capillaries. Registration marks were placed on each transparency for alignment

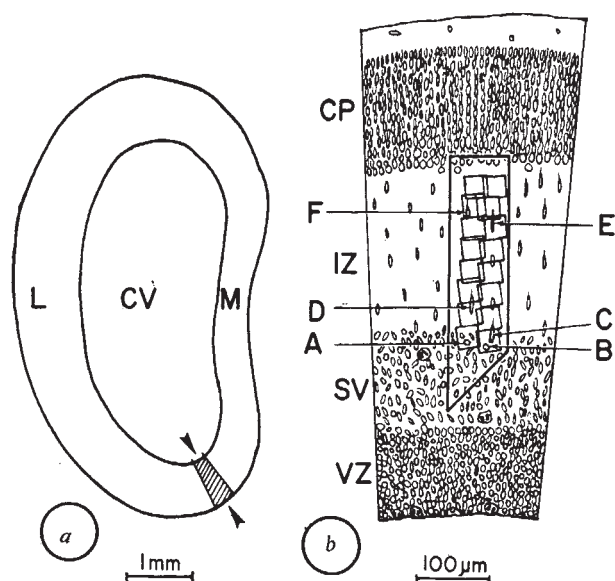


Fig. 1a Outline of a coronal section across the occipital lobe of a 58-d-old monkey embryo. The cerebral ventricle (CV) is relatively large at this stage and the lateral wall (L) is much thicker than the medial wall (M) on which the incipient calcarine fissure is slightly indented. The shaded area between the arrowheads indicates the position of the block of tissue that was processed for electron microscopy. **b**, Drawing of a 1 μ m thick, toluidine blue-stained section across the entire area shaded in (a). The cerebral wall from the ventricular surface at the bottom to the external surface at the top of the drawing consists of ventricular zone (VZ), subventricular zone (SV), intermediate zone (IZ) and cortical plate (CP). The area outlined by the rhomboid was cut serially for electron microscopy. Overlapping squares represent individual fields photographed for the reconstruction on photomontages. Arrows A to F point to the positions of six cells reconstructed in this study.

purposes. A representative electron microscopic field from which cell outlines were traced is illustrated in Fig. 2a. Considerable human judgement is involved in the choice of valid cell contours, and we thought it not worthwhile to attempt to automate this step.

Computer analysis

The cell and nuclear contours on the serial section transparencies were digitised as follows. The registration marks on successive transparencies were carefully aligned with pre-marked registration indicators on the digitiser to establish the x and y axes, and the height of the serial section above a reference level was dialled into the digitiser to provide elevation information (z axis). In the case of cells with complex shapes, more than one contour sometimes appeared on a transparency. Each contour on the transparency was traced with the cursor of the digitiser and the coordinates of selected points were recorded by the operator. The x-y coordinates of each point, the z coordinate of the transparency, and an identifying integer were transferred automatically to punched cards. Each closed membrane contour constituted a data set. Scaling information was recorded separately and used to convert the x-y-z data into absolute physical units.

The basic software for processing was prepared for an IBM 360/75 computer. The software reads the individual sets of data into memory, recognises multiple contours on a level, scales the data and computes the volume and surface area of each cell and nucleus as represented by the digital images. Registration is not necessary to obtain volume and surface area, but is necessary for graphic presentation.

Graphic software has also been written which gives the viewer the choice of observing the cell from any desired angle of rotation about the axis of the image (Fig. 2b-d). Standard Eulerian and projective transformations accomplish this efficiently. Presentation of the cell or nucleus as a three dimensional solid in space required the suppression of points hidden from the observer's eye. The requirement for a fixed number of points per contour is too strict a condition for the type of data considered⁸. A new method was developed which has no constraints on the number of points per contour or the number of contours per level. An arbitrary maximum can be set on the total number of points needed to specify the cell. The visibility algorithm depends only on the property of whether a point falls inside or outside the contour of visible points which preceded it. Visibility is determined data set by data set beginning with the first contour on the level closest to the observer and proceeding to the last on the contour furthest away.

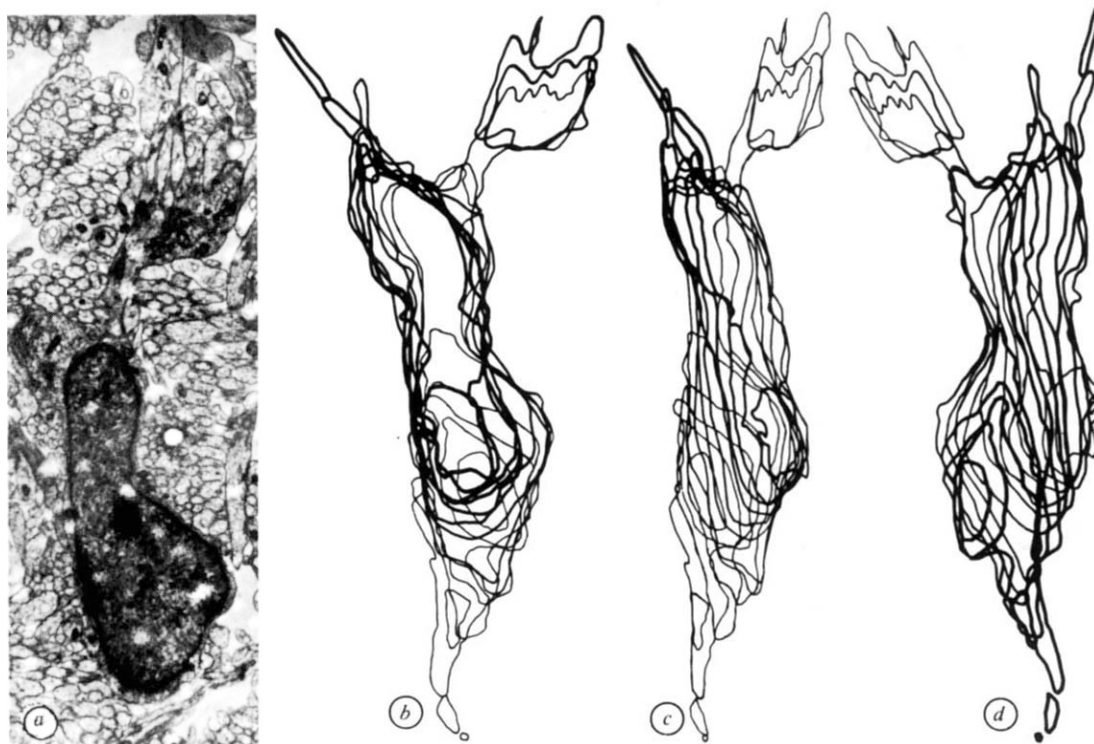


Fig. 2a Electron micrograph of cell C in serial section no. 83. Although the processes of all cells are in close apposition, it is relatively easy for the human observer to trace the irregular outline of a given cell. **b-d**, Cell C, as reconstructed from several fields, including that shown in (a). Only every ninth outline is represented, to make diagrams legible. In the three drawings, the cell is rotated on the axis running from pole to pole by 0°, 45° and 135°, respectively.

Considerable savings in computer time can be effected with a variation of the method. The intensity of each segment making up a contour is weighted according to its distance from the observer; nearer contours are reproduced in progressively thicker and darker lines, further ones are thinner and lighter (Fig. 2*b-d*). The effect achieved is similar to full hidden line suppression. To illustrate the savings achieved with this method, two trial cases each having 800 points of coordinate data representing 25 levels of contour data were processed using (1) full hidden line suppression, and (2) intensity weighting. Full hidden line suppression required 2.5 min of Central Processing Unit time on an IBM 360/75 computer compared with 0.5 min for intensity weighting. Therefore we used the latter mode of presentation in the preparation of the computer graphics presented here.

Three dimensional reconstruction

Of 24 cells reconstructed, six were examined in detail: two in the subventricular zone (Fig. 1*b*, cells A and B), two in the inner part of the intermediate zone (cells C and D) and two in the outer part of the intermediate zone (cells E and F).

volume though not in shape (Fig. 3*b, d* and *i-l*). The nucleocytoplasmic volume ratios and the cell surface area-volume ratios express numerically the relatively round compared with the elongated configurations of the cells (Table 1). The most interesting quantitative feature is the striking difference in cytoplasmic volume between cells E and F, which migrated the greatest distance, and cells C and D, which migrated less. If these four cells belong to the same postmitotic neuronal class, the data suggest considerable cell growth during migration. Alternative possibilities are that the cells are not statistically different in size or that cells C and D are smaller because they belong to a different population, destined for a different cortical layer¹⁴. Another important quantitative feature is the increase in surface area during elongation of the cells to bipolar shapes (compare cells A and B with C and D in Table 1) and a possible further increase in surface area as cells approach the cortical plate (cells E and F), an indication of net synthesis of cell membrane during migration. When more significant statistical samples are obtained new concepts may well emerge concerning the mechanisms and significance of cell movement in the developing central nervous system.

Figures 3*e-h* show that at the stage of cortical development under study, every migrating neurone examined is in contact with radially oriented fibres (compare refs 6 and 10). The leading processes of the migrating neurones have complex

Table 1 Quantitative cellular data

Cell	Surface area (μm^2)	Total volume (μm^3)	Ratio: area volume	Cytoplasmic volume (μm^3)	Nuclear volume (μm^3)	Ratio: nuclear volume cytoplasmic volume
A	140.1	155.0	0.904	44.7	110.3	2.47
B	177.7	239.6	0.741	50.5	189.1	1.83
C	266.7	200.9	1.33	75.1	125.8	1.68
D	212.0	179.8	1.18	63.5	116.3	1.83
E	305.4	264.7	1.15	121.3	143.4	1.18
F	235.9	239.8	0.984	103.2	136.6	1.32

From their positions and morphology as represented in random sections, we had suspected that cells A and B were subventricular (neuronal or glial precursors, possibly still proliferating), while cells C-F were postmitotic young neurones whose positions along the migration trajectory to the cortical plate should, in a general way, reflect their ages^{6,9}. Three-dimensional computer displays of the contours of cell C reconstructed from many sections, and viewed from three different angles are illustrated in Fig. 2*b-d*.

Drawings of all six cells, their nuclei and some contiguous bundles of fibres of relatively small calibre are shown in Fig. 3. These were made manually to scale directly from the tracings, with frequent reference to the original electron micrographs. Comparison of Figs 2*d* and 3*e* shows that the manual and computer reconstructions give similar results.

Volumes and surface areas

The computerised method of cell reconstruction reported here represents a departure from the slower and more laborious wax plate procedure adopted in earlier electron microscopic studies^{9,12,13}. It requires less time and labour, and provides a set of quantitative data of biological interest (Table 1). Although the sample of data is small, it suggests certain trends. Of the two subventricular cells, B has a much larger nucleus, which seems to be in a different class from those of the other cells, and we suspect both from this measurement and its electron microscopic appearance that cell B has just entered mitosis. The other nuclei resemble one another in

forms, reminiscent of the appearance of cells observed directly during migration in tissue culture¹¹. Leading processes commonly extend along more than one radial fibre (Fig. 3*e* and *h*). They terminate within the intermediate zone and in the sample of 24 cells, they have not been seen to extend into the cortical plate or through it to the external surface of the cerebrum. The cell shape (Fig. 3*g*) suggests that the leading processes are plastic, some withdrawn and others extended further as the cell migrates toward the cortex; if the number and form of the leading processes were fixed, this cell would be unable to advance beyond the fibre bundle that lies across the migration path and passes in front of one apical process and behind the other. Trailing processes were followed in some instances to their tapered terminations (for example, cell E) or to a point where they pass from the serially cut tissue (for example, cell C). It is also noteworthy that the rounded subventricular cells A and B, which do not seem to be migrating, likewise contact radial fibres (Fig. 3*a* and *c*). Such cells were not examined in the earlier studies^{6,10}, and it is not clear from this small sample whether or not subventricular cells usually relate to radial fibres.

Further prospects

Investigators may have some difficulty in estimating their computer requirements in relation to the complexity of their biological data. On the one hand, a mini-computer such as the PDP-11/05 with graphic accessories would permit the recording and display of a set of serial section contours but would allow only limited image rotation or data storage for large complex cells. On the other hand, a computer of the

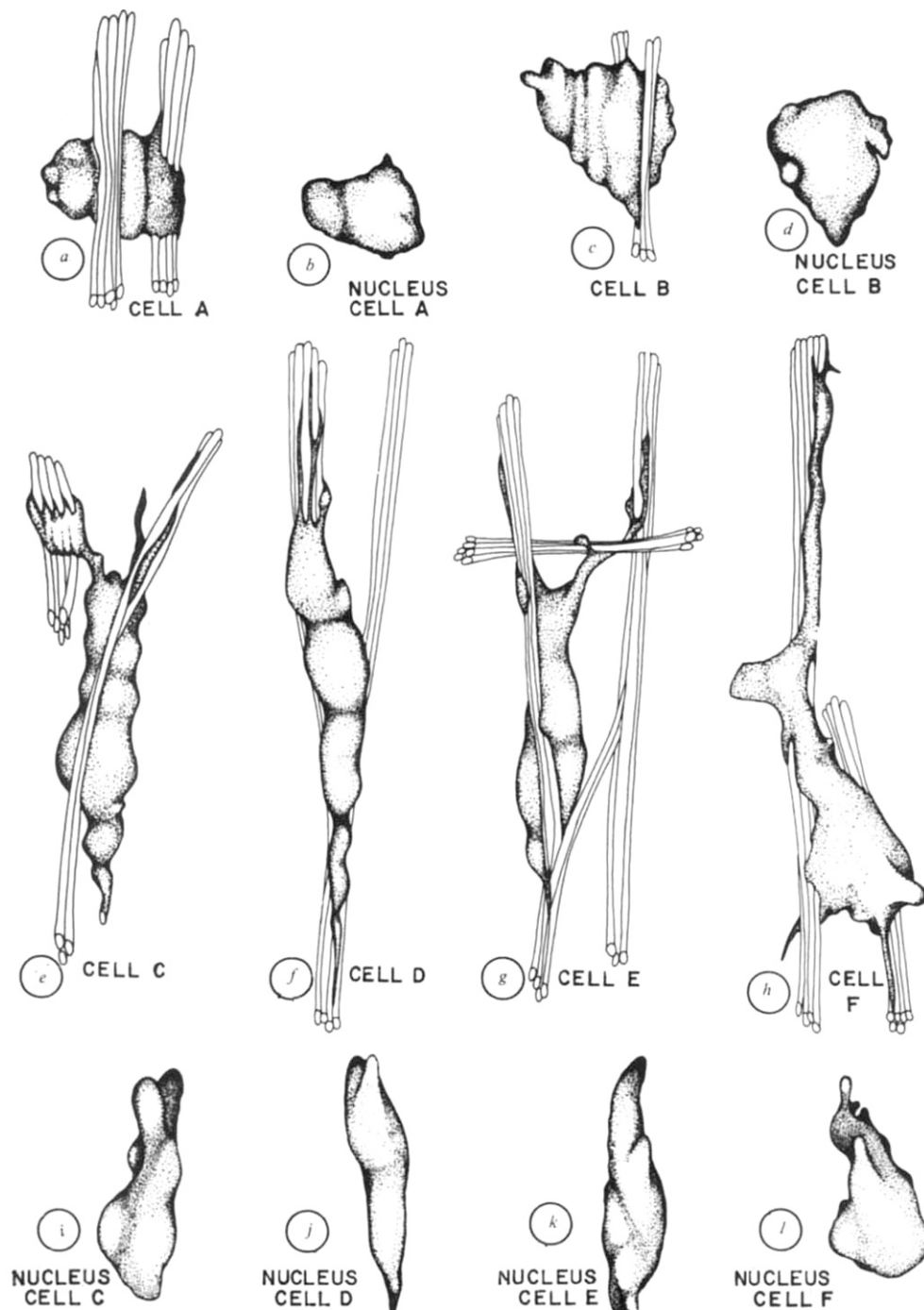


Fig. 3 a, c, e - h represent reconstructions of cells A to F respectively. Cells were drawn with the aid of superimposed outlines of cell profiles in serial sections at different levels traced onto transparent Mylar sheets. Some of the fibres which lie in contiguity with the migrating cells were also traced and reconstructed but most of the neighbouring cells and processes were omitted. b, d and i-l Represent similarly reconstructed nuclei of cells A to F, respectively.

IBM 360/75 type which we used has a larger capacity than we needed. Its greater storage capability will be necessary, however, to handle more complex issues such as spatial relationships among several cells that are changing their positions relative to one another in the developing brain. It will be needed also for analysis of the shapes and relationships of mature neurones, particularly for quantitative plotting and three dimensional display of synaptic inputs.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Was Jupiter the protosun's core?

THE differences between Jupiter and conventional stars are apparently due solely to its small mass. Therefore in looking for possible means of formation of the Solar System it is natural to attempt to establish the origin of the Jupiter-Sun binary system. The other planets could be just a by-product of the evolution of this system.

I shall start from the hypothesis that the close binaries form as a result of the rotational-exchange instability of the protostar (my unpublished work). This hypothesis can be formulated as follows.

Immediately after the collapse of the prestellar cloud, convection begins in the protostar formed¹. The initial differential rotation is replaced in a zone of convective mixing by uniform rotation (mixing can be caused by an instability of the differential rotation proper during the onset of quasihydrostatic equilibrium in the protostar). Because of the transfer of angular momentum, the centrifugal force can exceed the gravitational force in the outer layers. These layers will then become separated, forming a massive ring rotating around the protostar. A fast (adiabatic) loss of mass from the convective zone (the politrope index being 1.5) produces an increase in its size². As a result, the transfer of mass on to the ring, just as in the later stages of close binary evolution, proceeds on a 'dynamic' time scale until the convective envelope is totally lost³. The ring is unstable, breaking up and collapsing into one or several bodies at some stage.

In this way a multiple system is formed, and the mass ratio of its components should thus depend on that of the convective zone and of the stable core in the protostar. The fraction of the total mass involved in convection depends critically on the density of the prestellar cloud, but it should generally be larger the smaller is the mass of the protostar⁴. The accounts for the observed decrease (ref. 5 and my unpublished work) of the number of binaries with a short period ($P \lesssim 5-7$ d) and a small component mass ratio ($q < 0.8$) as one goes from B to F stars. Apparently the masses of the convective envelope and of the stable core in protostars forming close binaries with F components are, on the average, the same. The systems here have a $q \approx 1$.

With less massive protostars, systems with $q < 1$ should appear again (my unpublished work) but here the more massive component is formed by the lost matter. The loss of a larger mass releases a larger angular momentum J contained in the protostar. The system period increases at a fixed total mass, $P \propto J^2(1+q)^6 q^{-3}$ becoming longer than $5-7^d$. Such systems are difficult to find.

When convection extends throughout the protostar, practically all the initial mass should become transferred on to the object formed by ring condensation.

Indeed, convection penetrates into the star from outside, so that fast mass transfer begins, as a rule, before convection has reached the centre. Matter involved in convection is lost continuously so that the protostar retains only a small fraction of its initial mass at the time when the centre regains radiative stability after becoming convective.

The major factor determining the completion of transfer

is apparently the cooling of the protostar remnant caused by its fast expansion and radiation of energy. Nonvolatile components condense, forming in the protostar remnant a rocky core (and probably bodies orbiting in the outer rarified zone of the remnant).

Thus the above process of breakup of the protostar and of the mass exchange between its fragments results in the formation of a fairly wide system consisting of a newly formed massive star and the remnant of the protostar, its mass being much less than the initial mass. We now have a star with a satellite, that is with a planet.

Turning now to the Solar System, it is natural to assume Jupiter to be such a remnant of a protostar. This provides an easy explanation for the origin of the terrestrial planets and for their great difference from the massive Jupiter. These planets condensed and acquired angular momentum from the matter streaming from Jupiter—the protostar (the primary component)—to its satellite—the Sun (the secondary component).

This matter carries some angular momentum and therefore forms a rotating disk around the secondary. Keplerian motion in the disk is upset by friction transferring angular momentum to the disk's periphery. From here, the matter is thrown out by centrifugal force which carries away the momentum excess. The matter from the disk's interior is deposited on to the secondary⁶.

The gas dynamics of such a rotating disk have not been studied in detail but it is evident that the physical parameters should vary over a wide range, from thousands of K and tens and more atmospheres in the stream flowing from the primary⁷ and in shock waves generated by its motion, to hundreds of K and fractions of an atmosphere far away from both components. Within such a wide range one can easily envisage the realisation of the conditions necessary for the production of some chemical compounds and of their condensates which are needed for the formation of the terrestrial planets^{8,9}.

As for the planets beyond Jupiter, they could have formed from fragments of already sufficiently dense matter which remained from (or was thrown out in) the collapse of the ring separated from the protosun. The majority of the fragments coalesced and/or dispersed in collisions with one another and the parent bodies. The remaining fragments could have acquired arbitrary orientation of axial rotation (Uranus).

Afterwards, when the disk rotated around the Sun giving birth to the terrestrial planets, the loss of excess angular momentum from it resulted in the matter concentrating beyond the Jupiter-Sun system. Part of the matter was captured by the massive fragments-planets, while another part (just as in the inner disk but at a lower temperature) could condense to form planets similar in many respects to those of the Earth group, as well as comets (Oort's cloud).

The reasons for the slow rotation of the Sun, just as in the case of close binary stellar systems (my unpublished work), should be looked for in the effect of the rotation of the primary on the magnitude (and even sign) of the angular momentum of matter streaming away from it¹⁰. If the equatorial velocity of the primary greatly exceeds the

orbital velocity then the streaming matter will not form a disk, and the secondary (the Sun) will increase in mass with almost no increase of the momentum of its axial rotation.

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Gamma rays from black holes

As a result of electron ion decoupling, the compression of interstellar matter falling on to an isolated black hole results in ion temperatures greater than 100 MeV as the matter approaches the Schwarzschild radius. Here, experimental pion production cross sections are used to calculate the rate of production of γ rays from this hot gas. A characteristic γ -ray spectrum is produced, which peaks at 18 MeV regardless of the mass of the black hole or the interstellar density.

Matter accreting on to a black hole may be heated to very high temperatures and radiate γ rays^{1,2}. The accretion of matter onto a black hole can be treated as a hydrodynamic flow if a weak magnetic field is carried with the accreting gas^{1,3}. In the hydrodynamic model the accreting gas is rapidly compressed as it falls inwards, causing a significant fraction of the gravitational potential energy to be converted into thermal energy. Moreover, if the mass of the black hole is not too large ($\leq 10^4 M_\odot$) the accreting gas radiates away only a small fraction of the available thermal energy^{1,2}, so the compression of the accreting gas will be very nearly adiabatic. The temperature of the accreting gas at a distance r from the centre of the black hole is given by:

$$\theta(r) \simeq 0.5 \theta_0 (r_i/r)^3 (\gamma-1)^{1/2} \quad (1)$$

where γ is the average adiabatic index, r_i is the distance at which the gas begins to fall inwards, and θ_0 is the temperature of the gas as it begins to fall. The distance r_i will be given by⁴:

$$r_i = (2GM/c^2) (m_p c^2 / \gamma \theta_0) \quad (2)$$

where M is the mass of the black hole and m_p is the proton mass (assuming that the interstellar medium is predominantly hydrogen).

The temperature of the accreting gas near the gravitational radius, $r_g = 2GM/c^2$, depends markedly on whether the gas is ionised when it begins to fall inwards. If the gas is not initially ionised, then the temperature of the accreting gas does not get hotter than about 10^9 K (ref. 2). The gas near a black hole may, however, be ionised with $\theta_0 \sim 1$ eV (ref. 3), in which case (using a one-temperature approximation and assuming an average adiabatic index of 13/9 when the electrons become relativistic) there is a gas temperature of 10^{11} K near the Schwarzschild radius². The electron-ion coupling time, however, turns out to be much longer than the radial infall time so that it is more appropriate to use different temperatures and different adiabatic indices for the ions and electrons. As the ions are non-

relativistic at temperatures less than 100 MeV $\gamma = 5/3$ can be used, and the ion temperature is¹:

$$\theta_i(r) \simeq 0.3 m_p c^2 (r_g/r) \quad (3)$$

Thus, the ion temperature exceeds 100 MeV as the accreting gas approaches the gravitational radius. The ion density is¹:

$$n(r) = 6.10^{12} n_0 (r_i/r)^{3/2} \quad (4)$$

Considering the change in adiabatic index for the relativistic electrons, they approach a temperature of about 10 MeV near the gravitational radius if $M \leq 10^4 M_\odot$. The ion-electron Coulomb coupling time for $r < 100 r_g$ is $> 10^6$ s, which is far longer than the radial infall time $\lesssim 10^{-3}$ s, so the ions and electrons are indeed uncoupled. This is not altered by the presence of a turbulent magnetic field¹ if there is approximate equipartition of the magnetic field energy and the gas kinetic energy. The turbulent magnetic field tends to heat the ions at the expense of infall kinetic energy. The validity of the hydrodynamic approximation depends only on the smallness of the proton cyclotron radius relative to scales of interest.

At temperatures in excess of 100 MeV proton-proton collisions result in copious pion production, and γ rays will be produced because of the decay $\pi^0 \rightarrow 2\gamma$. This process is the main contribution to the γ -ray luminosity of black holes.

The rate of π^0 production in a hot gas with proper density n is $(n^2/2) \langle \pi_0 \rangle \bar{\sigma} v(\theta)$; where $\langle \pi_0 \rangle$ is the average number of π^0 's produced per collision, and σ is the pion production cross section. The quantity $\bar{\sigma} v(\theta)$ is related to the laboratory pion production cross section $\sigma(E)$ by:

$$\bar{\sigma} v(\theta) = \iint \sigma(E_L) v_L f(\mathbf{p}_1) f(\mathbf{p}_2) d\mathbf{p}_1 d\mathbf{p}_2 \quad (5)$$

where v_L is the laboratory velocity and f is the thermal distribution. Numerical evaluation of equation (5), using the experimental pion production cross sections⁵, and a relativistic Maxwell distribution, provides a curve of $\bar{\sigma} v$ against θ (Fig. 1). Knowing the ion temperature and density as a function of r the production of pion γ rays can be calculated as a function

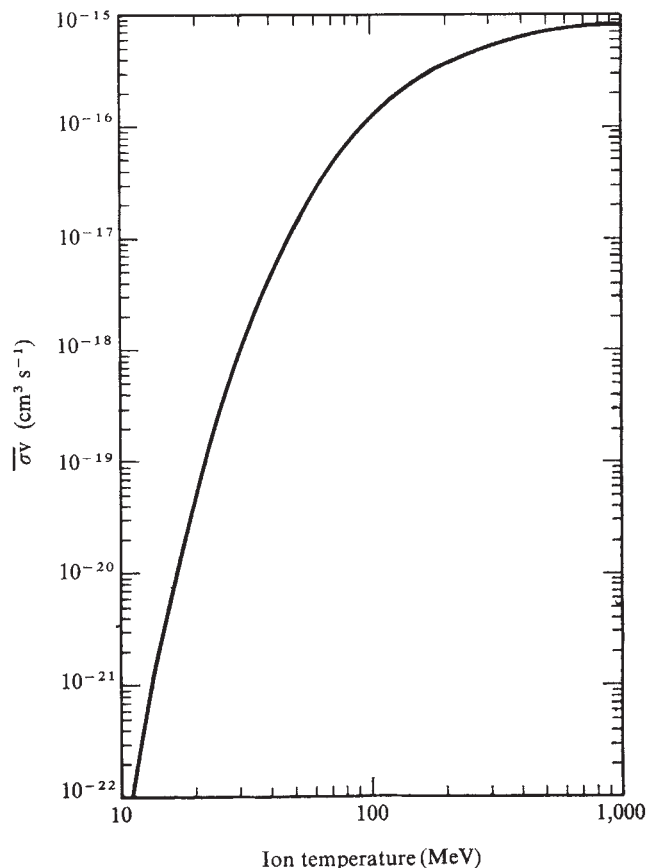


Fig. 1 Relativistic thermal average of σv , where σ is the cross section for the total pion production.

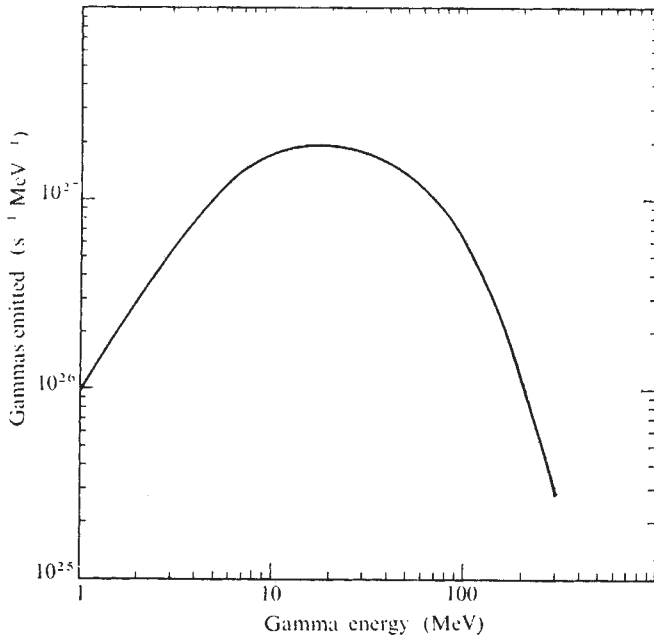


Fig. 2 Differential γ -ray spectrum from a $10 M_{\odot}$ black hole in a region of density $= 1 \text{ cm}^{-3}$, and $\theta_0 = 1 \text{ eV}$.

of r . The total rate of emission of γ rays resulting from spherical accretion is:

$$\dot{N}_{\gamma} = \int_{r_g}^{\infty} \langle \pi^0 \rangle n^2 \bar{\sigma} v(\theta) \left[1 + \frac{(\cos \psi - V/c)/(1 - V/c \cos \psi)}{2\pi r^2 dr} \right] (6)$$

where V is the radial infall velocity and ψ is the half angle of the radiation 'escape cone'. Numerical evaluation of the right side of equation (6), using the values of $\bar{\sigma} v(\theta)$ shown in Fig. 1 and equation (3) and (4) gives:

$$\dot{N}_{\gamma} \approx 3 \times 10^{26} (M/M_{\odot})^3 (1 \text{ eV}/\theta_0)^3 n_0^2 \text{ s}^{-1} \quad (7)$$

These γ rays have energies of the order of 20 MeV, so that the γ ray luminosity resulting from spherical accretion is:

$$L_{\gamma} \approx 10^{22} (M/M_{\odot})^3 (1 \text{ eV}/\theta_0)^3 n_0^2 \text{ erg s}^{-1} \quad (8)$$

This is larger than the γ -ray luminosity resulting from bremsstrahlung emission², but it is small compared to the total luminosity of the black hole, which results from the synchrotron radiation of visible light¹. It may be larger than indicated in equation (8) if matter is falling onto an isolated rotating black hole because of the accumulation of matter in orbits near r_g . Accreting disk models in binary systems⁶, have a much lower luminosity γ -ray because the accreted material has time to radiate a significant fraction of its energy, thus invalidating the adiabatic approximation.

The spectrum of γ rays emerging from the accreting gas will be determined by: the energy spectrum of the π^0 s as observed by a comoving observer; the Doppler shift resulting from the radial infall of the gas; and the gravitational redshift. More than half of the γ -ray emission comes from the region $r_g < r < 2r_g$, so the gravitational red shift is an important effect.

The γ -ray spectrum in a comoving frame which results from the energy spectrum of the π^0 s, can be calculated to sufficient accuracy by noting that for laboratory energies of less than 4 GeV almost all pion production occurs through an $N^*(1236)$ intermediate state. Thus, in order to calculate the γ -ray spectrum in a comoving frame only the γ -ray spectrum in the rest frame of an $N^*(1236)$ need be known. It can be obtained⁷ by convoluting the spectrum in the N^* rest frame with the relativistic thermal Doppler spectrum $\phi(\omega) = (2\omega)^{-1} \gamma(\omega) \exp[(m_{N^*} c^2/\theta_1) \gamma(\omega)]/K_1(m_{N^*} c^2/\theta_1)$; where $\gamma(\omega) = (\omega/\omega_0 + \omega_0/\omega)/2$ and ω_0 is the γ -ray energy in the N^* rest frame. If ω_1 is the γ -ray energy in the comoving frame then the frequency measured by a distant observer is:

$$\omega_{obs} = \omega_1 [(1-v/c)/(1+v/c)]^{1/2} (1-r_g/r)^{1/2} \quad (9)$$

(We have neglected the angular dependence of the Doppler

shift because the escape cone light of sight is nearly radial when the infall velocity is largest.) The spectrum obtained by integrating the contributions from all radii has a width of ~ 80 MeV, and falls off approximately as E^{-3} for $100 \text{ MeV} < E < 300 \text{ MeV}$ (Fig. 2).

The spectrum (Fig. 2) is universal in the sense that its shape is independent of the mass of the black hole or the interstellar density. Thus, if many black holes are present in the Universe some kind of anomalous feature could be expected in the γ -ray background in the neighbourhood of 10 MeV. The spectrum from black holes in our Galaxy would have a peak at 18 MeV; the spectrum from black holes in other galaxies would peak below 18 MeV because of cosmological redshift which lowers the peak in the 'background' spectrum to ~ 10 MeV. An anomalous feature in the γ -ray background at about 10 MeV has, in fact, been reported at a level of about $10^{-4} \gamma \text{ s cm}^{-2} \text{ sr}^{-1} \text{ MeV}^{-1}$ (refs 8 and 9).

If this anomaly results from isotropic emission from external galaxies with $30 M_{\odot}$ black holes, then about 10^{10} black holes per galaxy are required, assuming equation (7) for the γ -ray luminosity and $n_0 = 100 \text{ cm}^{-3}$. As mentioned, higher γ -ray luminosities may occur, thereby decreasing the number of black holes required. In our Galaxy, upper limits on the emission above 15 MeV from the galactic centre¹⁰ indicate that as many as 10^7 $30 M_{\odot}$ black holes may be in that vicinity if $n_0 = 100 \text{ cm}^{-3}$.

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Do black holes really explode?

THE creation of particles out of the vacuum will occur in regions of space-time where the metric is changing rapidly. Theoretical discussions of this process encounter some interpretational difficulties, however, because the concept of 'particle' is only well understood in Minkowski space. Nevertheless, in some simple cases, for example with the metric of homogeneous cosmologies, or of black holes of the Kerr and Schwarzschild type, the existence of a global timelike Killing vector allows a very plausible extension of the Minkowski space definition of particle. A number of exact results may then be proved. One of these results (ref. 1, and C. J. Isham and J. G. Taylor, personal communication) states that there is no creation of massless particles in the exterior region of a Schwarzschild black hole, which is the static end state reached as a result of spherically symmetric gravitational collapse. This result is not valid for a Kerr (rotating) black hole for essentially classical reasons².

Nevertheless, during the collapse of a spherically symmetric object, the metric near the surface will be changing rapidly on a time scale of $\tau \approx 10^{-5} (M/M_\odot)$ s, so that on general grounds the production of massless particles with energy of order h/τ is expected. Many of these particles will escape from the surface of the object and reach distant observers. The energy removed in this way will slow up the collapse and may even prevent it completely, thus causing the collapsing object to 'explode' in a burst of radiation. Exact calculations of this explosion are not possible with present theory, which does not enable the back reaction of the particle creation on the metric (a quantum gravitational correction) to be evaluated. A rough idea may be obtained, however, by treating quantised fields in a given classical background metric, and estimating the amount of particle emission as the collapse proceeds.

If we assume that the production rate is more or less constant during the collapse, the surface will have a luminosity L_0 , but a distant observer will see a rapidly fading luminosity as the retreating surface falls towards the event horizon:

$$L(t) = L_0 \exp(-t/\tau) \quad (1)$$

Particle creation will only be important for objects with $M < M_\odot$, and it follows from equation (1) that in this case the radiation will be emitted in a flash with a duration of much less than a microsecond. The spectrum of radiation will be given (roughly) by the Fourier transform of equation (1), that is, proportional to $v/(v^2 + v_0^2)$ where $v_0 = (2\pi\tau)^{-1}$. This is peaked around $v = v_0$ so that a distant observer might approximate this spectrum with that of a blackbody with a Planck spectrum $v/[\exp(hv/kT) - 1]$, corresponding to a temperature

$$T \approx h v_0/k \approx 10^{-6} (M/M_\odot) \text{ K} \quad (2)$$

where k is Boltzmann's constant. This result has already been obtained in another way³.

Treating the collapsing object as a blackbody radiator with temperature T for the duration of the 'flash', the energy emitted per unit time will be about $A\sigma T^4$, where σ is Stefan's constant, and A the surface area, which will not be greatly different from that of a final black hole of the same mass, that is, $16\pi G^2 M^2/c^4$. Consequently, the total mass loss by the object as a result of massless particle emission will be in the region of

$$(16\pi M^2 \sigma) \times (hv_0/k)^4 \approx hc/MG \quad (3)$$

with a numerical coefficient on the right hand side involving only small powers of 10. This result may be written as a fractional mass loss

$$\Delta M/M \sim (\text{Compton wavelength of object/Planck length})^2.$$

Evidently, black hole explosions with $\Delta M \approx M$ will only occur for an object the Compton wavelength of which is comparable with the Planck length, that is for masses of about 10^{-4} g and densities of 10^{94} g cm⁻³. This is just the region where quantum gravitational effects also become important. Indeed, this result is not surprising because in order to reverse the collapse, the back reaction of the created particles on the dynamics of the collapse needs to be appreciable, and this can only occur when quantum corrections to gravity become important.

Equation (3) is confirmed by the detailed calculations of Zel'dovich and Starobinskii⁴, who have treated the problem of massless particle creation in the context of anisotropic cosmological models. They have verified the formula $h/c^3 t^4$, originally deduced on dimensional grounds, for the energy density of created particles at a time t before collapse to a singularity. If a collapsing anisotropic single object is treated as a region of an anisotropic universe, this formula can be applied to obtain the total created particle energy-density over a time scale of order τ to obtain

$$(h/c^3)\tau^{-4} \times (\text{volume object}) \approx hc/MG$$

as before. Because of the additional tidal forces present in anisotropic collapse, spherically symmetric collapse would not be expected to lead to a greater particle production than this.

The conclusions of this simple treatment of particle production around collapsing objects seems to be in conflict with a recent result by Hawking⁵, who claims that black holes as large

as 10^9 gm would explode in massless radiation in about 0.1 s. Collapsing objects of this mass have an energy density only of the order 10^{-27} of that of quantum gravity fluctuations, so that this 'explosion' is more like a slow 'leak'. The e folding time for collapse is 10^{-29} s at this mass, so that Hawking's result implies that emission of massless particles continues to occur when the static limit of a Schwarzschild field is an exceedingly good approximation. It is not therefore clear what mechanism could be responsible for this emission (on the assumption of a reasonable definition of 'particle'). Any particles produced near the surface of a collapsing object will find it increasingly difficult to escape as the event horizon is approached. The last quantum ever able to reach a distant observer does so after an (asymptotic) time of only about 100 e folding times (10^{-27} s in this case)⁶. Hawking's result is, on the other hand, deduced on the assumption that the black-hole temperature given by equation (2) persists, unaffected by the collapse of the object, in the external asymptotic region, rather than fading out rapidly in accordance with equation (1). In our view it is a rather too literal interpretation of the concept of the 'temperature' of a black hole to apply it to emission processes in this way, and its use should be restricted to the discussion of absorption, as originally suggested by Bekenstein⁷.

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Further simultaneous hard X-ray and optical observations of Sco X-1

THE optical and X-ray emissions from Sco X-1 are thought to come from a hot plasma as small as or smaller than a white dwarf¹, but the energy source and the time variations of the radiation are not understood.

In our 1971 observations we found a positive correlation between the optical luminosity and the intensity of hard X rays at the optically enhanced phase of Sco X-1 (refs 2 and 3). Although the optical enhancement seemed to be a flare, a rather poor time resolution of the photographic observation prevented us from identifying it for certain. During a balloon flight on April 16, 1972, an X-ray enhancement was observed simultaneously with an optical flare, the latter being verified by photoelectric observation. With the result of another balloon flight on April 19, 1972, the hard X-ray spectra at several different B magnitudes are available for the study of the correlation between X-ray and optical emissions.

The instruments essentially the same as the one in the previous flight³, were launched on April 16 and 19, 1972, from Hyderabad, India. The balloon floated at the atmospheric depth of 2.9 to 3.0 g cm⁻² for about 4 h in each case. One of the counters in the April 16 flight malfunctioned, and its data were discarded.

Table 1 Results for Sco X-1 in selected periods

Date	Time (UT)	m_B	X-ray intensity at 30 keV ($\text{keV cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$)	Apparent* temperature	τ_{es}	Mass (10^{19}g)
May 1, 1971	2009–2018	12.6	0.060 ± 0.023	4.7 ± 0.7	8.6	1.7
May 1, 1971	2057–2106	12.4	0.12 ± 0.01	5.1 ± 0.3	9.9	2.0
April 16, 1972	2217–2225	12.5	0.12 ± 0.01	5.1 ± 0.4	10.0	1.8
April 16, 1972	2241–2246	12.3	0.20 ± 0.02	5.3 ± 0.5	10.7	2.3
April 19, 1972	2046–2117	12.7	0.035 ± 0.009	4.0 ± 0.7	11.0	1.3

* The plasma temperatures for the first four cases are obtained to be 3.6 keV, whereas 3.0 keV for the last case.

Simultaneously with the balloon flight, Sco X-1 was observed photographically with a 12-inch reflecting astrograph at the balloon launching station at Hyderabad and with an 8-inch astrograph at Nizamiah Observatory, and photoelectrically with a 48-inch reflector at Rangapur Observatory. All these optical data were consistent with each other in overlapping periods. Absolute X-ray intensities in three energy ranges, 17.3 to 22.3 keV, 22.3 to 27.1 keV and 27.1 to 31.8 keV from Sco X-1 on April 16 are shown in Fig. 1, with B magnitudes. They vary in parallel to each other. The optical luminosity increased by about 0.2 mag (around 2245 UT), while the X-ray intensity also increased by a factor of 2. In Fig. 1 we also show the apparent temperature that is derived by fitting the

X-ray spectrum in the energy range 20 to 40 keV to the exponential spectrum. In the previous papers^{2,3} the apparent temperature was defined by fitting the observed X-ray spectrum to the thermal bremsstrahlung spectrum with the energy-dependent Gaunt factor. The apparent temperature found here is lower than that obtained with the method used before.

The hard X-ray spectra obtained in selected periods of the two flights are compared with those of the previous flight on May 1, 1971, in Fig. 2. The B magnitudes, X-ray intensities and apparent temperatures on the assumption of the exponential spectrum in these selected periods are listed in Table 1. A positive correlation between X-ray and optical emissions is clearly observed in the optically bright phase, whereas the apparent temperature shows only a weak positive correlation, in agreement with our previous result^{2,3}. On the other hand, rather strong positive correlations have been reported for lower X-ray energies⁴⁻⁶.

The observed features can be interpreted in terms of thermal bremsstrahlung from a hot, semi-opaque plasma. Thermal bremsstrahlung X rays are subject to Compton scattering by hot electrons. So the spectrum of X rays is considerably modified in the energy range 1 to 10 keV (refs 7–9), whereas only minor modification is predicted in the energy range above 20 keV, as is revealed by a theory of photon transport (unpublished work by J. N.). In the optical region free-free absorption suppresses the intensity of radiation emitted. Essential features of the spectrum are dictated chiefly by the optical depth for electron scattering τ_{es} , and also to some extent by the electron density and the shape of the plasma. For comparison with our experimental data, we have drawn theoretical X-ray spectra for several optical depths (S. H., M. M. and I. K., unpublished). The calculated spectra are in essential agreement with the observed ones in non-flaring and flaring periods, respectively, if the total mass of plasma particles is increased by about 40%. In all cases the plasma temperatures are found to lie in the range 3.5 to 3.8 keV.

Properties of Sco X-1 obtained by the simultaneous X-ray and optical observations are summarised as follows:

(1) The X-ray intensity in the energy range 20 to 40 keV shows a positive correlation with optical luminosity in the bright phase of Sco X-1. In general the amplitude of X-ray variability is much larger than that of optical luminosity.

(2) The apparent temperature of Sco X-1 derived from the spectrum in the energy range 20 to 40 keV does not appreciably change in comparison with considerable spectral variations in the energy range 1 to 10 keV. The plasma temperature does neither appreciably change during our observations.

(3) Flare activity is associated with an increase of the total mass of a hot plasma.

It should, however, be noted that on a few other occasions less steep spectra and higher intensities have been observed in optically less bright periods. Among these cases properties of Sco X-1 in the period of $m_B > 12.9$ may be different from those we are concerned with. An exceptional result was found by Miyamoto *et al.*¹⁰ who observed an intensity twice as high as ours in the flaring period and a less steep spectrum for $m_B = 12.6$. According to Uhuru observations, the correlation between X-ray and optical emissions in some rare periods

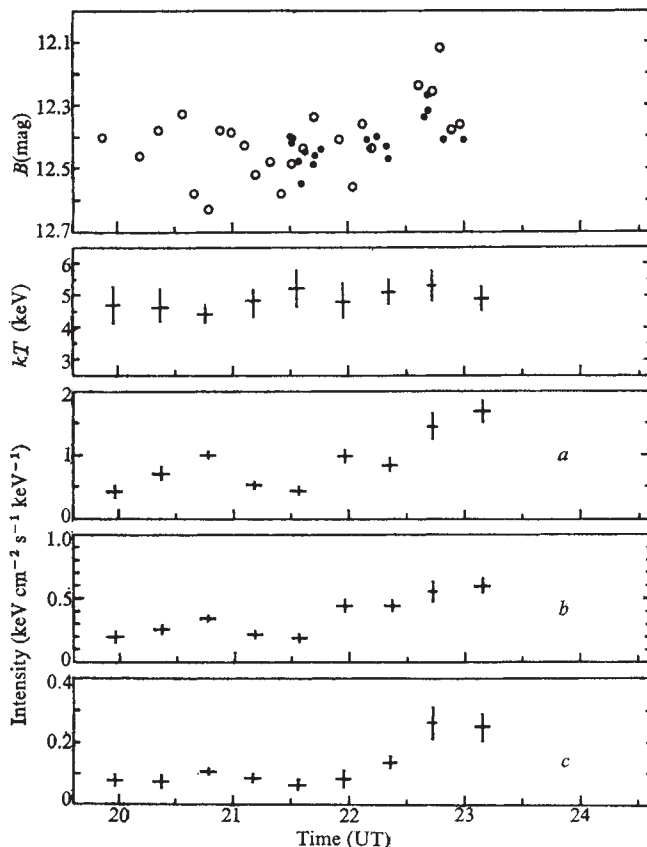


Fig. 1 Time variations of X-ray and optical emissions observed on April 16, 1972. B magnitudes indicated by open circles and black dots were obtained by the photographic observation with a 12-inch reflector and by the photoelectric observation with a 48-inch reflector. The apparent temperature kT is derived by fitting the observed spectrum to the simple exponential form. The X-ray intensities in three energy ranges are obtained by subtracting the background counting rates and correcting for the effective area of the counter and the atmospheric absorption. *a*, 17.3 to 22.3 keV; *b*, 22.3 to 27.1 keV; *c*, 27.1 to 31.8 keV.

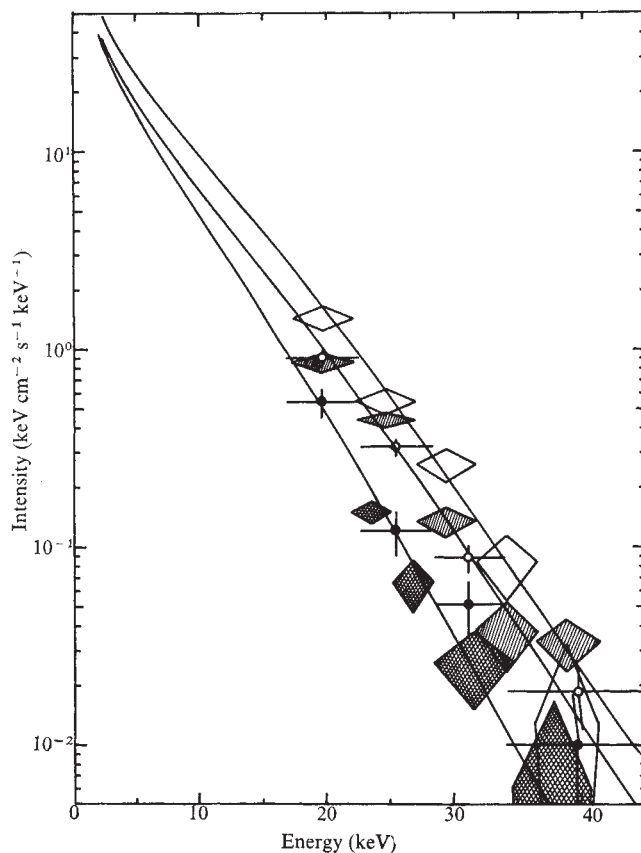


Fig. 2 Hard X-ray spectra observed in five selected periods. For comparison theoretical spectra are given for three periods in the 1972 flights with the values of plasma parameters given in Table 1. May 1, 1971: ●, 2009 to 2018 UT, $B=12.6$; ○, 2057 to 2106 UT, $B=12.4$. April 16, 1972: open diamond, 2217 to 2225 UT, $B=12.5$; hatched diamond, 2241 to 2246 UT, $B=12.3$. April 19, 1972: cross-hatched diamond, 2046 to 2117 UT, $B=12.7$.

is different from that observed in most periods (H. Gursky, unpublished). Thus further comprehensive research will be demanded in order to construct a convincing model for any case of Sco X-1.

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Origin of neutron star magnetic fields

PULSARS are generally thought to be rapidly rotating neutron stars which have poloidal magnetic fields with intensities consistently in the range 10^{12} – 10^{13} gauss (ref. 1). Most of them are believed to evolve from main sequence stars with original masses of >1.4 – $8 M_{\odot}$. Evolutionary computations^{2,3} indicate that stars in this mass range develop a dense core of carbon and oxygen in which carbon begins to burn when the temperature reaches about 3×10^8 K and the density about 3×10^9 g cm⁻³. The rapid production of energy during this carbon-burning stage drives almost the entire core into convection for an extended time of the order of 10^{11} s (ref. 4), with convective velocities of the order of 10^4 cm s⁻¹ (ref. 5), provided that the rate of cooling through the convectively driven 'Urca process' is sufficiently high to stabilise carbon burning and to prevent the core from detonating and dispersing. If this sequence is a reasonable approximation to reality then the existence of neutron star pulsars suggests that carbon burning is, in fact, stabilised until electron capture, or some other process, causes part of the core to collapse to a neutron star²⁻⁷.

The origin of pulsar magnetic fields with nearly uniform intensities¹ is a matter of some interest. If a main sequence star with dimensions of $\sim 10^{11}$ cm contains a poloidal field, of about 100 gauss in its interior, then conserved magnetic flux during collapse to neutron star dimensions of $\sim 10^8$ cm will produce a magnetic field of the order of 10^{12} gauss. This line of argument is usually relied on in discussions of the origin of the magnetic fields of pulsars. We have argued (not yet published) that turbulent transport and mixing of the magnetic field, during the convective carbon-burning stage, will dominate its behaviour in the core. In this case the simplest notions of conserved magnetic flux do not apply, the accepted line of

reasoning fails and the final magnetic field of the collapsed neutron star is not a unique descendant of the field which the star possessed while on the main sequence.

Also in this connection, Ruderman and Sutherland⁸ have suggested that turbulent convection during the carbon-burning period will produce an equipartition magnetic field with an energy density equal to the kinetic energy density of the convecting fluid. The final state reached by a turbulent, conducting fluid and magnetic field has not, however, been resolved, so this suggestion must be considered speculative. Although we feel that such speculation is along the right lines, it contains a difficulty which, to us, seems significant. According to Ruderman and Sutherland⁸ an initial seed magnetic field, B_s , of spatial scale R , is rigorously frozen into the fluid and stretched and tangled by the turbulence until it attains an equilibrium intensity, B_{eq} , such that the energy density of the magnetic field is equal to the energy density of the turbulence. A convective core with radius $R \sim 10^8$ cm, and with an average density of 10^9 g cm⁻³, produces an equipartition magnetic field with $B_{eq} \sim 10^9$ gauss. It follows, however, that in the absence of some specially contrived fluid velocity which is a function of the magnetic field, the final field will have a spatial scale, δ , given by

$$\delta \sim R \sqrt{(B_s/B_{eq})}$$

Using the discussed values, $\delta \sim 3 \times 10^3 \sqrt{B_s}$ cm; even with a seed field as large as 10^5 gauss, $\delta \sim 10^6$ cm. Collapse through two orders of magnitude in radius, to the dimensions of a neutron star, then results in the neutron star magnetic field having a scale of $\sim 10^4$ cm and an intensity of $\sim 10^{13}$ gauss. Therefore, the surface magnetic field of a neutron star consists of some 10^4 patches of magnetic field with intensities of 10^{13} gauss, with randomly varied polarities. Each patch has a magnetic flux of $\sim 10^{21}$ gauss cm². The mean net flux through a hemisphere of the neutron star is then expected to be $\sim \sqrt{(10^4) \times 10^{21}}$, which is $\sim 10^{23}$ gauss cm². This is about an order of magnitude below what is inferred to be characteristic of pulsars¹. Furthermore, this process of magnetic field amplification leads to a large statistical spread in the net magnetic moments of neutron stars. The uniformity of neutron star magnetic moments, as inferred from the observed properties of pulsars, seems to indicate that the magnetic fields are produced with large spatial scales as well as with uniform intensities.

We suggest that convection during the carbon-burning stage (before collapse to a neutron star) in a massive, rapidly and differentially rotating, degenerate stellar core, leads to the production of a large scale magnetic field within the core itself, through the action of a hydromagnetic dynamo. Solutions of the hydromagnetic dynamo equations⁹⁻¹¹ use a dimensionless number, N , known as the dynamo number^{12,13}:

$$N = \gamma \Gamma R^3 / \eta^2$$

where γ is the rate of nonuniform rotation, Γ is essentially the cyclonic component of the convection in a rotating body, R is the spatial scale of the dynamo region and η is the magnetic diffusivity. We have calculated (not yet published) the conditions which will produce a quadrupole like magnetic field confined to the convecting core of a star. A magnetic field will be generated if the fluid motions are such that N falls within the range of $\sim 3,400$ to $\sim 12,000$. In a turbulently convecting core, transport and diffusion of the magnetic field will be dominated by turbulent mixing, and so we use the magnetic diffusivity, $\eta \sim 0.1 \nu_t \lambda$, where ν_t is the turbulent fluid velocity and λ is the scale of the large turbulent eddies^{14,15} (which we take to be about a pressure scale height). Setting ν_t equal to the average differential fluid velocity¹⁵ in the convecting and differentially rotating core, a magnetic field is produced when the convection extends over 5–10 pressure scale heights, as is the case in the carbon-burning core. The e-folding growth time of the magnetic field intensity is calculated to be of the order of days ($\sim 10^6$ s) in the carbon-burning core. This is short compared with the time over which carbon burning occurs⁴, so the field has sufficient time during this stage of stellar evolution to reach a saturation value, which will be determined by the dynamics of the fluid flow. We have estimated the saturation value of the

mean magnetic field throughout the rapidly rotating degenerate core by equating the Coriolis force on the convection, to the magnetic field stress. We find the mean of the poloidal and toroidal components to be $\leq 10^9$ – 10^{10} gauss. This estimate should only be taken as an approximation. It has been calculated by neglecting density variations throughout the dynamo region and by using only the crudest hydrodynamic considerations. The final state of the magnetic field will depend on quantities not yet calculated, such as the fraction of the core which actually collapses to a neutron star. With this quantitative caution in mind, however, we suggest that such a dynamo magnetic field—with a spatial scale which is that of the core itself, and a poloidal intensity of the order of 10^{12} – 10^{13} gauss after collapse to neutron star dimensions—is the progenitor of pulsar magnetic fields.

We consider elsewhere¹⁶, further dynamical consequences of this hypothesis.

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Lewisian age for the Scardroy Mass

Rocks of Lewisian aspect have long been recognised east of the Moine Thrust in Sutherland, Ross and Inverness. They were correlated with the Lewisian west of the Moine thrust on petrological grounds¹ and were originally considered to be unconformably overlain by Moine rocks². Sutton and Watson^{3,4} at first questioned this correlation, but later accepted the view that rocks derived from the Lewisian basement are exposed at

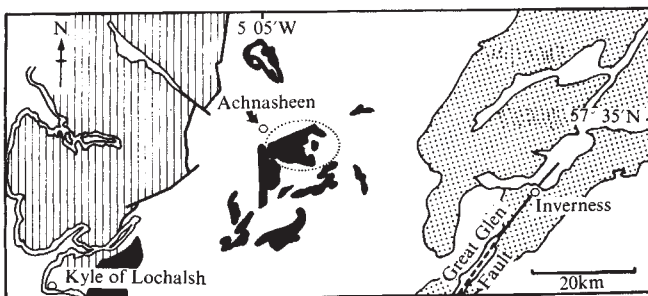


Fig. 1 Sketch map showing Lewisian 'inliers' (black) originally recognised by the Geological Survey within the area of Moine rocks (white). The Scardroy area, south-east of Achnasheen, is enclosed by a circle of dots. Vertical lines, Lewisian and younger rocks west of the Moine thrust; dots, Old Red Sandstone.

Table 1 Rb-Sr whole rock analytical data

Sample no.	Description* and locality	$^{87}\text{Rb}/^{86}\text{Sr}\dagger$	$^{87}\text{Sr}/^{86}\text{Sr}\dagger$	Rb (p.p.m.) $\ddagger$	Sr (p.p.m.) $\ddagger$
MT-1	Leucocratic, strongly banded, fine-grained gneiss. Pl, Qz, Kf, Bi, Ap. North side of Loch Beannacharain: grid ref. NH229523.	0.859 ± 0.012	0.7383 ± 0.0002	84	285
MT-5	Mesocratic, strongly banded, medium-grained gneiss. Pl, Qz, Ep, Bi, Kf, ore, Ap. North side of Loch Beannacharain: grid ref. NH233520.	0.191 ± 0.006	0.7110 ± 0.0003	29	436
MT-9	Mesocratic, banded, medium-grained gneiss. Pl, Qz, Bi, Ep, Zo, Kf, Sph, Ap. Between Scardroy Lodge and Carn Mhartuin: grid ref. NH203526.	0.461 ± 0.006	0.7222 ± 0.0003	75	472
MT-10	Mesocratic strongly banded, fine-grained gneiss. Pl, Qz, Bi, Hb, Kf, Ep. North of Loch Beannacharain: grid ref. NH220520.	0.127 ± 0.006	0.7090 ± 0.004	19	427
MT-12	Mesocratic, weakly banded, coarse-grained, massive gneiss. Pl, Bi, Qz, Ep, Hb, Ap. North side of Loch Beannacharain: grid ref. NH223519.	0.055 ± 0.002	0.7069 ± 0.0005	19	988

* Kf, potash feldspar; Pl, plagioclase; Qz, quartz; Bi, biotite; Hb, hornblende; Ep, epidote; Zo, zoisite; Sph, sphene; Ap, apatite; ore, iron oxide minerals.

$\dagger$ Errors quoted at 95% confidence level. $^{87}\text{Sr}/^{86}\text{Sr}$ normalised to $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$.

$\ddagger$ Semiquantitative values to $\pm 10\%$.

Grid references refer to the 1-inch Ordnance Survey Map of Great Britain, Sheet 27, Strathpeffer.

Table 2 K-Ar analytical data

Sample no.	Description and locality	Mineral	K(%) $\ast$	Radiogenic ^{40}Ar ($\text{cm}^3 \text{g}^{-1} \text{STP} \times 10^{-4}$)	Radiogenic ^{40}Ar Total ^{40}Ar (%)	Age (Myr) $\dagger$
MT-2c	Melanocratic, banded, medium-grained, amphibolitic gneiss with feldspathic patches. Hb, Pl, Qz. North-east side of Meall Dubh: grid ref. NH232530.	Hornblende	0.61, 0.61, 0.62	0.1510	89.7	535 ± 26
MT-3	Melanocratic, massive, coarse-grained, patchy, amphibolitic gneiss. Hb, Pl, Gt, Bi, Qz. North side of Meall Dubh: Grid ref. NH227532.	Hornblende	0.74, 0.74, 0.74	0.1532	94.6	459 ± 22
MT-11a	Melanocratic, massive, coarse-grained, amphibolitic gneiss. Hb, Bi, Pl, Gt. North side of Loch Beannacharain: grid ref. NH222520.	Hornblende Biotite	0.55, 0.54, 0.55 7.26, 7.27, 7.26	0.1040 1.361	83.7 96.9	426 ± 20 420 ± 20
MT-11b	Massive, black, coarse-grained hornblende-biotite rock. Hb, Bi. Grid ref. NH222520.	Biotite	7.47, 7.48, 7.40	1.422	98.2	427 ± 10

$\ast$ The mean of the individual K determinations is used for the age calculations.

$\dagger$ Errors quoted at 95% confidence level.

Abbreviations as in Table 1; Gt, garnet. Grid references as in Table 1.

Scardroy and elsewhere in central Ross-shire (Fig. 1), and take the form of sheets tectonically intercalated in the Moine succession⁵.

In order to test this hypothesis, Rb-Sr whole rock analyses were carried out on a suite of typical gneisses from the Scardroy sheet (Table 1). $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were obtained on a 12-inch mass spectrometer, and Rb/Sr ratios were accurately determined by X-ray fluorescence⁶. Rb and Sr contents were estimated semiquantitatively only. The decay constant of ^{87}Rb was taken as $1.39 \times 10^{-11} \text{yr}^{-1}$. K-Ar dates of separated minerals are presented in Table 2. K was determined by flame photometry and ^{40}Ar by isotope dilution using an AEI MS-10 mass spectrometer. The decay constants for ^{40}K are $\lambda_{\beta} = 4.72 \times 10^{-10} \text{yr}^{-1}$ and $\lambda_{\epsilon} = 0.584 \times 10^{-10} \text{yr}^{-1}$. Full analytical details have been given elsewhere^{6,7}.

The Rb-Sr whole rock data yield an isochron age of $2,810 \pm$

120 Myr, with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7039 ± 0.0007 (Fig. 2). The scatter about the least-squares fitted line is not accounted for by analytical error alone and an increase of errors by a factor of two is needed to obtain a valid fit.

The data prove beyond any doubt that the rocks of the Scardroy sheet are, indeed, Lewisian. The actual age obtained is well within the age range for early Scourian rocks west of the Moine thrust, dated by Rb/Sr, U/Pb and Pb/Pb methods⁸⁻¹⁰. This age has been interpreted as the time of Scourian metamorphism. The rather low initial $^{87}\text{Sr}/^{86}\text{Sr}$ value is in general accord with much published (and unpublished) isotopic work^{8,10,11} suggesting that Scourian gneisses are not produced by remobilisation of substantially older (> about 200 Myr) sialic rocks with average continental Rb/Sr ratios.

The K-Ar dates for hornblendes and biotites (Table 2) are mostly within the typical range of mineral dates found in the

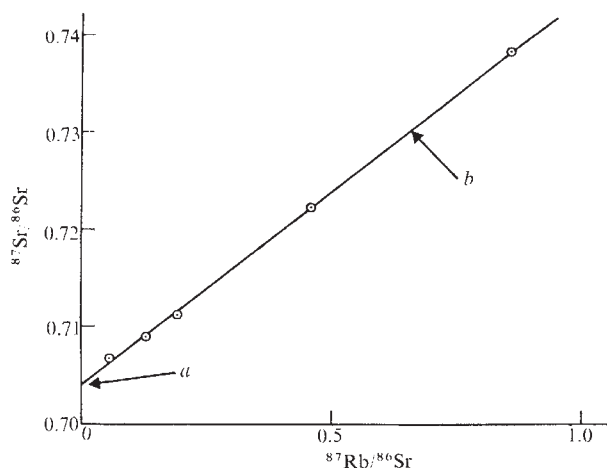


Fig. 2 Rb-Sr whole rock isochron plot for analysed samples. Errors are at 95% confidence level: a, 0.7039 ± 0.0004 ; b, $2,810 \pm 120$ Myr.

British Caledonides^{12,13}. Sample MT-2c yields a date of 535 Myr and may not have been completely degassed during the Caledonian metamorphism.

In the western Glenelg Lewisian inlier immediately to the east of the Moine thrust at Loch Duich, where Caledonian effects are not nearly as intense as at Scardroy, most hornblende and biotites still yield K-Ar dates of approximately 2,200–1,600 Myr, whereas slightly further east in the eastern Glenelg Lewisian inlier at Loch Duich biotites and hornblendes already yield typical Caledonian dates (Moorbath, unpublished work).

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Solid tides recorded with 1 m interval mechanical strainmeter

A TENSIONED-QUARTZ strainmeter 1 m in length has successfully recorded solid tidal strain variations in the Cooney Observatory. The designed is based on the 10-m quartz catenary strainmeter already in use at Cooney¹ Vitreosil quartz rods (2-mm diameter) linked with 4-mm diameter

Vitreosil hooks comprise the standard. This is tensioned by a brass beam-balance mounted to the hornfels by two $\frac{3}{8}$ inch expanding bolts. A single $\frac{3}{8}$ inch expanding bolt supports the other end of the quartz standard. Mounted within the fixed body of the beam balance is the coil housing of a Tesa GT10 inductive displacement transducer. The sensor armature is connected to the balance arm, (rotating on flexure strips), entering the transducer coil without contact (see ref. 2 for details).

A low impedance output (sensitivity $500 \text{ mV } \mu\text{m}^{-1}$) is provided from the Tesa electronic unit by a buffer amplifier. A Multiscript Model 3 chopper-bar recorder was used in these tests, sensitivity corresponding to 200 nm full scale deflection. The trace can be resolved visually to 2 nm (4% of maximum tidal amplitude). Zeroing is achieved with the visual readout and the electrical output facility provided in the Tesa unit.

The chopper-bar recorder was used for long period signals, sampling at 6 s intervals. Short period records of noise level were taken on an analogue pen recorder. (The use of a shorter interval of quartz results in a considerably higher resonant frequency than for the 10-m instrument³.)

Installation involves drilling three holes in the rock and setting the expanding bolts which support the covers and

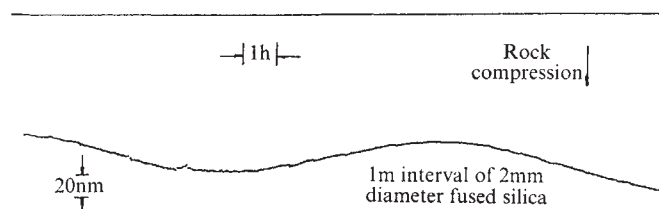


Fig. 1 Section of actual record obtained with the 1-m tensioned-quartz instrument.

instrument ends. The instrument is then mounted and set approximately. Fine adjustment of the length finally is made by varying the balance mass to obtain a zero reading on the electronic unit. The task takes no more than an hour; no special skills are needed.

We selected a site to make best use of available conditions. The Lower Cooney tunnel, (other strainmeters reported are placed in an upper complex) is 316 m below the topographical surface. A 12-m long, narrow chamber, situated 300 m into and parallel with this drive has been sealed off at both ends, entry being through a sealed door at one end. In addition, a 10 cm thick expanded polystyrene box surrounds the entire meter. Temperature variations of 4 mK occur within the insulated cover with a noticeable regularity of 20 to 30 min cycles. (Measurements were made with a thermistor bridge, resolution of $100 \mu\text{K}$.)

Figure 1 shows part of the record obtained with the 1 m interval strainmeter, taken 6 d after completion of the installation. The drift over this interval is 2 parts in 10^9 per hour. A drift curve for the same meter, installed on the thermally controlled measuring base⁴, is given in Fig. 2, showing that the wall-mounted unit is stabilising in an expected manner.

The 10 Hz bandwidth noise level is no greater than 0.4 nm peak-to-peak, giving a signal to noise ratio of better than 40 dB when the amplitude of the tide is large.

The amplitude of the tide rises to about 50 nm. This is greater than expected at this site for a 1 m interval. Records from 10 m interval instruments in the Upper Cooney complex indicate that a maximum strain of 3 parts in 10^8 is normal. Comparison between this 1 m instrument and a 10 m quartz catenary are also situated in the same chamber, will enable us to investigate this departure in due course.

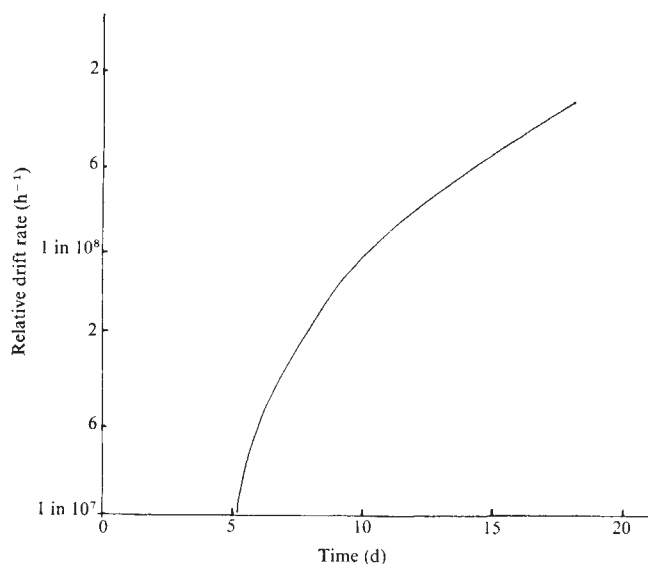


Fig. 2 Drift curve of 1-m instrument mounted in test base³ (relative to stabilised steel).

Also under development, in the same chamber, is a 1-m interval floor-mounted strainmeter that is coupled to the Earth by brass plates resting on a thin bed of sand. One plate carries a modified Tesa GT10 probe which has its armature supported by two thin phosphor-bronze diaphragms. Clamped onto the other plate, in a horizontal attitude, is a 1 m length of Vitreosil tube (outside diameter 16 mm). The tube is connected to the transducer by a quartz wobble-pin, completing the measurement loop. So far we have been unable to achieve satisfactory continuous records with this instrument because of excessive instability. But because of the advantages of plates on sand in terms of rapid mount stabilities⁴ and ease of installation, we will continue to investigate this design. Separate studies of mounts⁵ and experience with a 10-m quartz-tube instrument using bolted rock joints¹ suggests the method should perform satisfactorily.

Initial drift being a function of mounting rather than the standard, has an absolute value independent of gauge length. A 1-m instrument will, therefore, require a longer settling time than a 10-m unit.

Future research should be aimed at obtaining a closer thermal coupling between the instrument and rock and that it be located in deep undisturbed rock. Records obtained from a 1-m corehole meter (possibly using a rock core as a standard) may bear a closer relationship to the phenomena being studied than any records obtained to date.

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Obsidian hydration profile measurements using a nuclear reaction technique

AMBIENT water diffuses into the exposed surfaces of obsidian, forming a hydration layer which increases in thickness with time to a maximum depth of 20–40 μm (ref. 1), this layer being the basic foundation of obsidian dating^{2,3}.

We have used the resonance at a ^{19}F energy of 16.45 MeV (0.83 MeV centre-of-mass energy) (ref. 4) in the nuclear reaction $^1\text{H}(^{19}\text{F}, \alpha\gamma)^{16}\text{O}$ to measure directly the hydration profiles of obsidian samples. This technique has already been used to measure the hydrogen concentration profiles in lunar samples and other solids to depths up to 0.4 μm with a resolution of 0.02 μm (refs 5 and 6). A second strong resonance at 17.64 MeV is encountered in extending these measurements to greater depths⁴, but its contribution can be unfolded from the data.

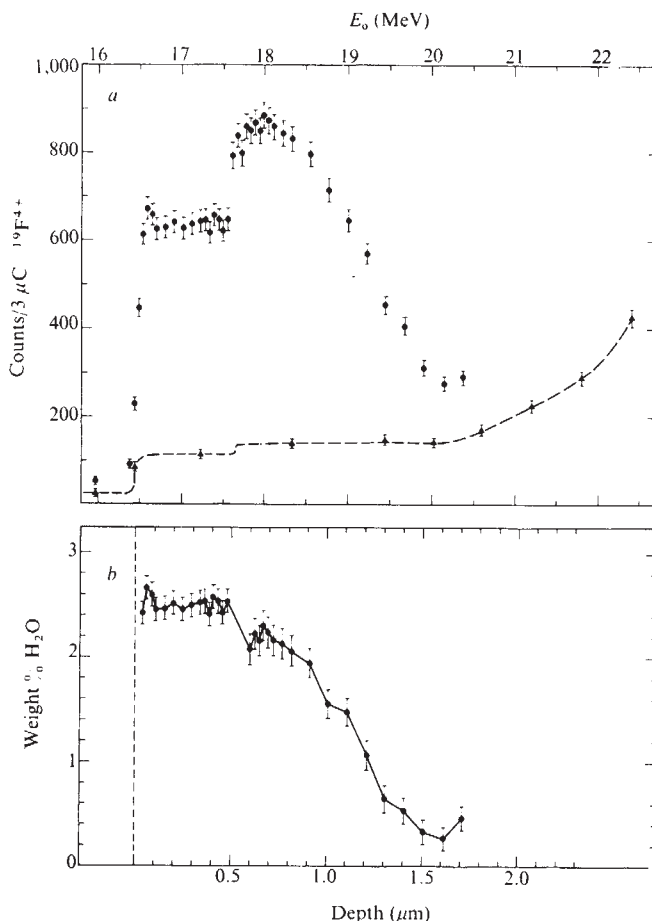


Fig. 1 Raw data for sample 5 (●) and for an unhydrated (0.3% H_2O) (▲) sample. Error bars indicate uncertainties arising from counting statistics. The background problem for $E_0 > 20$ MeV is illustrated by the profile for the unhydrated sample; a corresponding increase in counting rate occurs for all samples at $E_0 > 20$ MeV. To minimise this background, the interpolated data for the unhydrated sample were subtracted point-by-point from the data for sample 5. Then, for data points at energies $E_0 > E_R = 17.64$ MeV, the effects of the second resonance at E_{R2} were unfolded in accordance with equation (3) in the text: K_2 multiplied by the number of counts at $E_0 - \Delta E$ was subtracted from $Y_1(E_0)$ to obtain the unfolded hydration profile (b). The error bars in b indicate the propagation of errors contributed by uncertainties arising from counting statistics in the data for sample 5 and the unhydrated sample, and the unfolding of the second resonance. Using a calibration sample of known H content, the counting rate was converted to a weight % H_2O scale; using tabulated stopping powers⁷, the E_0 scale was converted to a depth scale. The segment of maximum H_2O gradient (for b, for example, at 1.2 ± 0.2 μm) was interpreted as corresponding to the thickness of the optically observed hydration band.

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Table 1 Summary of the quantitative data derived from the measured hydration profiles (see Fig. 1)

Sample no.	Source, identification	% H ₂ O by weight	Thickness of hydration band (μm) ¹ H(¹⁹ F, αγ) ¹⁶ O measured profile	Thin-section technique
1	Big Obsidian Flow, Newberry Craters, Oregon. Artificially hydrated at 75° C for 1 d	2.11 ± 0.14	0.089 ± 0.020	—
2	Big Obsidian Flow, Newberry Craters, Oregon. Artificially hydrated at 75° C for 2 d	2.08 ± 0.13	0.113 ± 0.020	—
3	Big Obsidian Flow, Newberry Craters, Oregon. Artificially hydrated at 75° C for 4 d	2.45 ± 0.14	0.14 ± 0.02	—
4	Big Glass Mountain, Medicine Lake Highlands, California. Collected August 1963—fresh surface exposed then	2.16 ± 0.12	0.19 ± 0.02	0.39*
5 (Fig. 1)	Big Obsidian Flow, Newberry Craters, Oregon. Exposed surface	2.5 ± 0.1	1.2 ± 0.2	1.2 ± 0.2
6	Amapa, Nayarit, Mexico. Artefact	1.9 ± 0.5	1.55 ± 0.5	1.4 ± 0.2
7	Amapa, Nayarit, Mexico. Artefact	2.15 ± 0.15 1.56 ± 0.05	0.9 ± 0.2 1.77 ± 0.27	1.7 ± 0.2
8	Amapa, Nayarit, Mexico. Artefact	2.3 ± 0.1	1.8 ± 0.3	1.8 ± 0.2
9	Amapa, Nayarit, Mexico. Artefact	2.65 ± 0.10 2.1 ± 0.1	0.53 ± 0.1 1.82 ± 0.25	2.1 ± 0.2
10	Borax Lake, California. Chipping waste	2.8 ± 0.1	1.8 ± 0.5	0.7 ± 0.5†

* Thickness of hydration layer was not measured directly using the thin-section technique but was estimated from the known time of exposure of the fresh surface.

† Large uncertainty because of poor sample preparation.

The '% H₂O by weight' was assigned to the plateau value of the H₂O concentration profile for the sample, with uncertainties determined by finding the highest and lowest horizontal lines that could be construed to comprise the plateau. The thicknesses of the hydration bands given by the ¹H(¹⁹F, αγ)¹⁶O measured profile were obtained by taking the midpoint of the segment of maximum H₂O gradient on the sample profiles. The uncertainty is determined by the depths corresponding to the endpoints of the segment of maximum concentration gradient. The two values of H₂O content and hydration-layer thickness, as measured by the ¹H(¹⁹F, αγ)¹⁶O technique, for samples 7 and 9 may reflect the existence of two plateaux in their profiles. A possible systematic error introduced by our calculated stopping powers into the measurements of both hydration-band thickness and H₂O content has not been taken into consideration; the calculated stopping power has roughly a 5% uncertainty. Thicknesses of hydration bands, optically determined by microscopic examination of thin sections from the same obsidian samples, are given for comparison.

If a monoenergetic beam of ¹⁹F⁺ ions with energy E_0 slightly above the resonance energy E_R is focused on an obsidian sample, the ions gradually slow down because of electronic collisions (the rate of energy loss being characterised by the stopping power dE/dx of the obsidian) until at a depth X_R the resonance energy E_R is reached. At resonance, the nuclear reaction—in particular the γ-ray production—proceeds at a rate proportional to the hydrogen concentration in a thin layer at X_R . Assuming that all hydrogen present in the hydration layer is incorporated as H₂O, the reaction therefore proceeds at a rate proportional to the water concentration at X_R .

As the beam penetrates still deeper into the sample than X_R , the ¹⁹F energy decreases below the resonance energy. Because the nonresonant cross section on either side of the resonance energy is negligible compared with the cross section at resonance, the hydrogen outside the layer at X_R contributes negligibly to the total reaction yield. Since the stopping power dE/dx is very nearly constant in the relevant range of ¹⁹F energies (16 to 22 MeV) (ref. 7), the depth X_R is related to the incident beam energy E_0 by

$$X_R = (E_0 - E_R)/(-dE/dx) \quad (1)$$

(dE/dx is a negative quantity). Thus, measuring the γ-ray production rate as E_0 is varied gives a direct indication of water concentration as a function of depth in the target.

Since there actually are two resonances (at $E_{R1} = 16.45$ MeV and $E_{R2} = 17.64$ MeV), three situations are possible: $E_0 < E_{R1}$, $E_{R1} < E_0 < E_{R2}$ and $E_{R2} < E_0$.

If $E_0 < E_{R1}$, the ¹⁹F ions are gradually slowed down in the obsidian and the ion energy never reaches either resonance. Hence, the γ-ray production for $E_0 < E_{R1}$ is very small and defines the background level below the resonance.

If $E_{R1} < E_0 < E_{R2}$, the ¹⁹F ions will lose energy in the obsidian, and will eventually reach E_{R1} at a depth X_{R1} and

produce γ-rays at a rate proportional to the hydrogen concentration at X_{R1} . That is:

$$Y_t(E_0) = Y_{R1}(E_0) = K_1 W(X_{R1}) \quad (2)$$

for $X_{R1} < \Delta E/(-dE/dx)$, where K_1 is a measurable proportionality constant determined by the width and cross section of the resonance and the stopping power dE/dx , $Y_t(E_0)$ is the total γ-ray production rate, $Y_{R1}(E_0)$ is the rate of γ-ray production due to the resonance at energy E_{R1} as a function of E_0 , $W(X_{R1})$ is the water concentration at depth X_{R1} , and $\Delta E = E_{R2} - E_{R1} = 1.19$ MeV.

If $E_{R2} < E_0$, the ¹⁹F ions will, after losing some energy in penetrating the obsidian, reach both resonance energies, but at different depths X_{R1} and X_{R2} . So,

$$Y_t(E_0) = Y_{R1}(E_0) + Y_{R2}(E_0) \\ = K_1 W(X_{R1}) + K_2 W(X_{R2})$$

where K_2 is a second proportionality constant differing from K_1 because of different resonance parameters. But since $X_{R2} = X_{R1} - \Delta E/(-dE/dx)$,

$$Y_t(E_0) = K_1 W(X_{R1}) + K_2 W(X_{R1} - \Delta E/[-dE/dx]) \quad (3)$$

for $X_{R1} > \Delta E/(-dE/dx)$. So for a given E_0 , the contribution of γ-ray counts related to the resonance at E_{R2} is proportional to the H₂O content at a shallower depth than X_{R1} , the depth corresponding to the contribution due to E_{R1} . At this shallower depth, however, ($X_{R2} = X_{R1} - \Delta E/[-dE/dx]$), the H₂O concentration has already been measured by the first resonance at E_{R1} using a lower ¹⁹F beam energy ($E_0 - \Delta E$). Thus, the contribution $K_2 W(X_{R1} - \Delta E/[-dE/dx])$ from the second resonance can be calculated from other data (the constant K_2 having been independently determined) and may be subtracted from $Y_t(E_0)$, leaving only $K_1 W(X_{R1})$, the contribution from the first resonance. In this way $W(X_{R1})$, the water concentration as a function of depth in the obsidian target, may be determined by measuring $Y_t(E_0)$ and applying this simple un-

Table 2 Comparison of water content (in percentage by weight) of hydrated and unhydrated obsidian samples from the same source

Sample location	Intrinsic unhydrated % H ₂ O	Hydrated saturation level % H ₂ O
Bodie Hills, California	0.21 ± 0.04	2.31 ± 0.20
Coso, California	0.23 ± 0.07	2.68 ± 0.25
Borax Lake, California	0.35 ± 0.09	3.32 ± 0.32
East Dago Valley, California	0.66 ± 0.06	3.47 ± 0.25

The unhydrated surfaces were freshly chipped from the interiors of obsidian samples, and thus had no significant exposure to ambient water. The hydrated samples all had hydration profiles whose saturation plateaux extended deeper than the 2 μm limit of our depth range. There is a monotonic relationship between intrinsic and hydrated water concentration levels; the higher the intrinsic concentration of water, the higher the final saturation level. This suggests a possible relationship between the factor which determines the intrinsic water content and the mechanism by which ambient water diffuses into obsidian.

folding procedure. With the present experimental configuration^{5,6}, γ rays are detected with an efficiency of 0.022, using a 7.6 cm $\times$ 7.6 cm NaI (TI) scintillation detector.

We made hydration profile measurements as follows on a number of obsidian samples with hydration bands less than 2 μm thick. Raw data (γ -ray counts per 3 μC of $^{19}\text{F}^{4+}$ against E_0) were taken over an energy range of 16 to 22 MeV. The beam current was sufficiently low that the hydrogen concentration was not perturbed, that is, subsequent measurements on the same samples gave virtually identical results. The counting rate (Y_i) was converted to water concentration by comparison with a standard chlorite sample of known H content, and other mineral standards.

A value of 2.4 g cm^{-3} for the density of hydrated obsidian, and values of stopping powers taken from Northcliffe⁷, were used to compute dE/dx for ^{19}F ions in the obsidian (~ -2.3 MeV μm^{-1}). Using this value of dE/dx in equation (1), the ^{19}F energy scale was converted into a depth scale. (The depth resolution is determined by the ratio of the resonance half width to the stopping power and is about 0.02 μm at the surface; energy straggling gradually reduces the resolution to about 0.04 μm at a depth of 2 μm . Because of background problems at higher energies, this technique is limited to depths of $\lesssim 2$ μm .)

The hydration layers of samples 1–4 (Table 1) were sufficiently narrow that the second resonance was not encountered and unfolding was unnecessary, so the raw data were plotted directly. But since the contribution of the natural linewidth of the resonance to the observed energy width of the hydration layer is significant for such thin layers, correction for this effect was made in order to obtain the measured values for the thickness of the hydration layer.

For samples 5–10, data from an unhydrated obsidian sample (water content uniformly 0.3%) were subtracted point-by-point from the raw data to minimise background problems for $E_0 > 20$ MeV. (For this reason, the zero point of the water concentration scale is displaced by 0.3%.) The profile was then unfolded (equation 3) to leave only the contribution from the resonance at 16.45 MeV. These reduced data were then plotted and were fitted with the calculated water content and depth scales. This procedure is illustrated for sample 5 in Fig. 1.

The detailed hydration profiles can be used to obtain information about the mechanism of water diffusion into obsidian and the factors which influence hydration. First, the general shape of the diffusion profiles agrees qualitatively with that espoused by Friedman and others^{1–3}, characterised by a saturated hydration plateau followed by a steep diffusion front, rather than the more conventional exponential profile suggested by Marshall⁸. Occasionally, however, the data suggest either a double plateau level or that the top of the hydration plateau has an appreciable slope, as in samples 7 and 9, which may indicate the existence of more than one

mechanism of water diffusion and binding as proposed by Ericson, MacKenzie, and Berger⁹.

Table 1 shows that the thickness of the hydration layer corresponding to the maximum gradient of water concentration obtained from our measurements agrees reasonably well with the layer thicknesses obtained from the optically measured thin sections. This seems plausible on theoretical grounds since the hydration band is visible under cross-polarised light because of stress birefringence, with the border between hydrated and unhydrated regions corresponding to the line of maximum stress². (Under ordinary light, the demarcation between hydrated and unhydrated glass is given by a dark line which is the line of maximum gradient of the water concentration².) Indeed, a set of tektite samples with measured H₂O distributions characterised by gently sloping exponential diffusion profiles rather than the steep diffusion fronts observed in hydrated obsidians, did not have visible hydration bands in thin section. (The tektites we measured all had exposure times of roughly 700,000 yr, according to K–Ar and fission track dates, and were recovered from laterite soils in South-east Asia. Two samples, of the splash-form type, were recovered in the early 1960s; a third sample, recovered in the later 1960s, is from the Muong Nong site (J. O'Keefe, private communication).)

Our resolution of 0.02 μm represents a significant improvement over the resolution of 0.1 or 0.2 μm achieved with optical techniques². The uncertainty in our measurements of the thickness of the hydration layer is limited by the finite slope of the concentration decrease for the layers 1–2 μm thick; it is not a limitation imposed by our technique (except where the spacing of data points has been wider than optimum), but by the actual profile itself.

Second, as a result of our measurements of H₂O concentrations in hydrated and unhydrated samples from the same source, it would seem that the higher the intrinsic (unhydrated) concentration of water in a given obsidian sample, the higher the final saturation level (Table 2). (Tektites have extremely low intrinsic water content compared with obsidians¹⁰; this possibly indicates differences in their internal structure, as proposed by Berger and Ericson¹⁰, which might explain the qualitatively different hydration profiles.) This correlation supports previous findings by Kimberlin¹¹.

Third, it seems that, for a set of samples from the same obsidian source exposed for equal times¹², the saturation concentration of water is weakly correlated with the thickness of the hydration layer (Table 1, samples 6–9).

Fourth, we observed a progressive increase in hydration depth with hydration time for the artificially hydrated samples (Table 1, samples 1–3). This type of experiment should prove particularly useful in investigating variations of hydration rate with chemical composition, since the hydration can be carried out under controlled conditions.

Using a similar technique with the resonance $^{23}\text{Na}(p, \gamma)^{24}\text{Mg}$ at 1.318 MeV (ref. 13), we have measured the sodium depth distributions to a depth of 1 μm in obsidian samples 1 and 4 to check a hypothesised ionic-exchange diffusion mechanism which predicts a sodium depletion in the hydration layer⁹. We found no significant variation of sodium content between the layer and the intrinsic unhydrated obsidian; however, we were limited by counting statistics to a detection threshold of about 10% variation in the Na content.

Resonant nuclear reaction techniques of this type may be extended to many other diffusion problems. What is required is a strong, isolated resonance which can be associated unambiguously with the diffusing agent under investigation. The technique provides a relatively nondestructive method of measuring diffusion profiles directly on a microscopic scale.

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Using artificial thermoluminescence to reassemble statues from fragments

THE thermoluminescence (TL) of marbles and limestones¹⁻³ from various quarries in Greece⁴ has been described. It had been hoped that features characteristic of each quarry would be found. The glow curves of samples as taken from the quarry are called natural thermoluminescence curves (NTL). These curves did not allow any determination of origin, because in nearly every case only a single peak was obtained.

Glow curves of artificial thermoluminescence (ATL) provide considerably more information. ATL glow curves were obtained by heating samples until the NTL was erased, then exposing them to X rays at room temperature, and subsequently heating them at a constant rate.

Another type of glow curve, the curve of mixed thermoluminescence (MTL), was obtained by exposing untreated samples to X rays at room temperature and subsequently heating them up at a constant rate. With this kind of glow curve the ATL curve is superimposed on the NTL curve. Most MTL glow curves displayed three peaks, and thus supplied five parameters for the identification of each sample:

(temperatures T_1 , T_2 , T_3 ; and intensity ratios I_1/I_2 and I_2/I_3). A table of all five parameters in material from 36 quarries has been compiled. In some of the quarries, significant differences could be detected between samples from widely separated blocks. The differences were more pronounced between blocks of different colours.

Because of the differences in the parameters of samples from any one quarry, it is not possible to identify unambiguously the origin of any piece of marble. The differences between samples from any one block are, however, small, suggesting that there could be a good possibility of determining whether separate fragments of marble are from the same statue or slab. Only small quantities of powder are available for investigations of archaeological fragments. A comparison of powders (~ 0.1 g) from adjacent holes showed that the MTL glow curves give identical peak temperatures, although the intensity ratios were sometimes different. This could be because of differences in the degree of heating of the material during the drilling process, which depends on the pressure exerted.

The results are consistent if the comparison is made from the ATL glow curves (Fig. 1a and b) which are not affected by any heating during drilling. Although these usually have only three independent parameters: T_1 , T_2 and I_1/I_2 they differ very little (about 5°C for the temperature and 5% for the ratios on powders from adjacent drilling holes).

From a consideration of several glow curves of the same sample mean values of the parameters can be obtained, together with their standard deviations (Table 1).

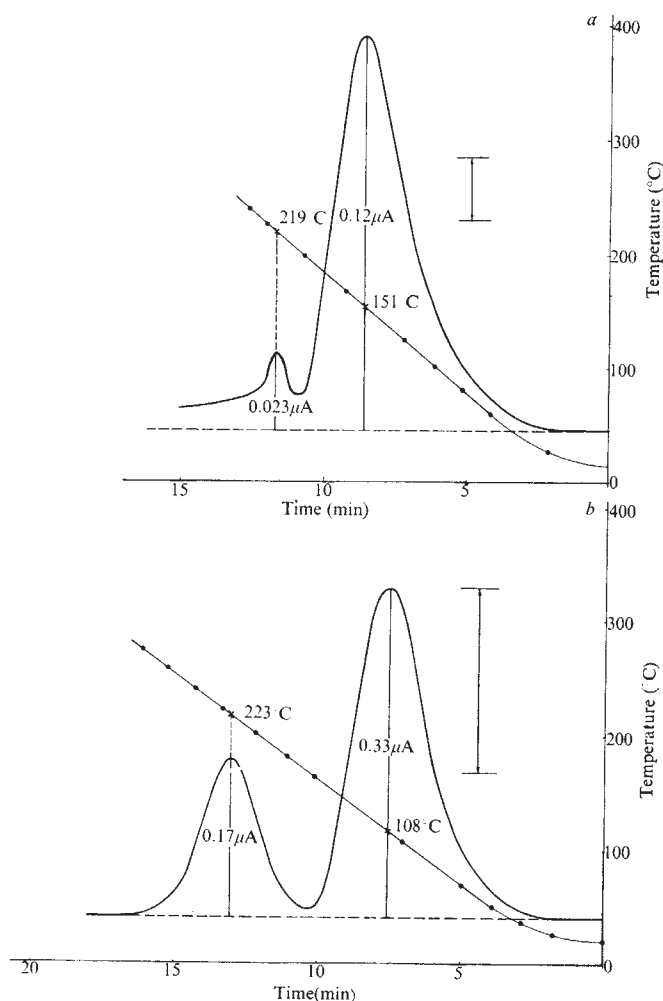


Fig. 1 ATL glow curves from two different blocks. The results were obtained on a chart recorder. Scale bars: a, 0.02 μA ; b, 0.2 μA . The dependence upon temperature is shown on both graphs.

Table 1 ATL curve parameters

	T_1 °C	T_2 °C	I_1/I_2
Fig. 1a	108 ± 5	223 ± 4	1.9 ± 0.1
Fig. 1b	151 ± 5	219 ± 7	5.1 ± 0.6

If the parameters are the same, it does not necessarily follow that the fragments belong to the same block, as they could simply have originated in the same quarry. If, however, two fragments have different parameters it can be said with a high degree of certainty that they do not belong to the same block.

This method was of great assistance in the restoration of statues from fragments found in a shipwreck near the island of Antikythera.

An extensive description of the method will appear, in English, in the *Praktika* of the Athens Academy.

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BIOLOGICAL SCIENCES

Mutant of bacteriophage T4D affecting expression of many early genes

IN T4 infections the mechanisms determining the time and rates of expression of early genes are not yet clearly understood. Although mutants which do not make late gene products or which are defective in DNA synthesis generally fail to turn off the synthesis of most early gene products, no mutations have been described which affect either the time of appearance or the initial rate of synthesis of proteins from more than a single early gene. This preliminary report describes the isolation and some of the properties of such a mutation. This mutation which maps between genes 52 and *t* defines a new regulatory gene.

When a lambda lysogen is singly infected at 37° C with a phage carrying temperature-sensitive mutations in both *rIIA* and in gene 42 (dCMP-hydroxymethylase¹), the infected cells quickly lose their ability to form a plaque at 30° C. After 25 min at the restrictive temperature only about 2% of the infected cells can still form a plaque at 30° C. It seemed possible that the survival of the *rIIA*⁻gene 42⁻ infected cells might be increased by an additional mutation whose effect would be to prevent the expression of many early genes. That is, if the normal development of the phage-infected cell was suspended at an early stage then the early absence of functional *rIIA* product might not be lethal². On shifting such infected cells to the

permissive temperature, normal development might then proceed as active *rIIA* protein was synthesised. According to this rationale, the survivors of the heat treatment (25 min at 37° C) would include phage which were genetically less sensitive to this treatment. Among mutants thus selected there might therefore also be mutants controlling the expression of early T4 genes.

The selection was done by singly infecting *E. coli* K112-12 (λ h) #3/S at 37° C with the non-mutagenised double temperature-sensitive mutant *tsDG12* (*rIIA*²)-*tsL13* (gene 42). After 25 min at 37° C the infected cells were filtered and resuspended in fresh media at 30° C. The infected cells were lysed at 50 min by the addition of chloroform. This enrichment procedure was repeated several times using the progeny phage from one cycle of infection as the input phage for the next cycle. The infected cells in the fifth cycle of enrichment were significantly more resistant to the temperature treatment than cells infected with the parental phage. Stocks were made from isolated plaques of several of these surviving phage and one such isolate was thoroughly examined. Although this phage still contained both of the parental markers, about 20% of the K112-12 (λ h) #3/S cells infected with it (by comparison with only about 2% for the parental phage) could still make plaques on *E. coli* B (su⁻) at 30° C after 25 min of infection at 37° C. This phage contained a new mutation in addition to the two parental markers. This mutation, *tsG1*, when separated from the two parental markers is itself a temperature-sensitive mutation. It seems to reduce the lethal effects caused by the initial absence of functional *rIIA* protein, but not the lethal effects due to the absence of functional gene 42 product (P42) (manuscript in preparation).

Figure 1 presents the results of two-factor crosses which show that *tsG1* maps in the region between *amH17* (gene 52) and *amtA3* (gene *t*). The mapping of *tsG1* to the right of *amH17* is consistent with the results of a three-factor cross between *tsG1* and the double mutant *amH17-ac*³. Most of the *ts*⁺-*am*⁺ recombinants (78 out of 103) examined were sensitive to acridine.

Reversion studies suggest that *tsG1* is a point mutation. In three isolated plaques of *tsG1* grown at 30° C revertants appeared at an average frequency of 1×10^{-6} . Experiments with one of these *tsG1* revertants showed that it was like wild type at both 30° C and 42° C in its growth in liquid medium and in its pattern of protein synthesis as seen on SDS-polyacrylamide gels (data not shown). This revertant was crossed to wild type and 870 progeny phage tested for their temperature sensitivity. No *tsG1* segregants were found. On this basis it is concluded that *tsG1* is a point mutation and that reversion can occur at the site of the original lesion or very close to it.

The mutants *tsG1* and *amH17* (gene 52) complement one another in a mixed infection of *E. coli* B^E at 42° C in the sense that the mixed infection begins to produce progeny at the same time

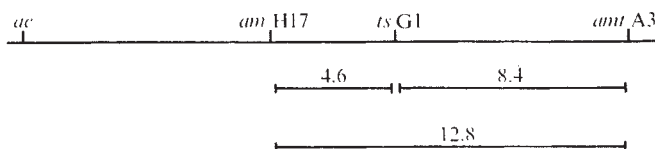


Fig. 1 Mapping by two-factor crosses. Log phase *E. coli* B^E grown at 37° C to 10^8 ml⁻¹ in M9S medium¹⁰ containing 0.2% casamino acids (Difco) were centrifuged and resuspended at 2×10^9 ml⁻¹ in fresh medium. Crosses were done at 30° C using a multiplicity of infection of five for each parent. After 3 min of adsorption the infected cells were diluted 10^4 into fresh medium and aerated by bubbling. Chloroform was added at 50 min to ensure complete lysis. Progeny were assayed on plates made of EHA bottom and top agar¹¹. Total progeny were assayed at 30° C using *E. coli* B₄₀ sul⁺ as indicator. Wild type recombinants from all crosses were selectively assayed on plates seeded with *E. coli* B^E and incubated at 42° C. The map distances given are 200 times the frequency of wild type recombinants scored in crosses between the markers indicated. *tsG1* and *amH17* each recombine with the terminal *rIIB* marker, *r73*, to about the same extent, giving a map distance of approximately 15 units.

as a wild type infection and not with the delay characteristic of either *amH17* or *tsG1* (data not shown). The pattern of protein synthesis seen in *amH17* infections (see below) is also quite different from that observed in *tsG1* infections. It is therefore very unlikely that *tsG1* is in gene 52.

In addition *tsG1* is not in the *t* gene for the following reasons. The *t* mutants specifically affect late events in T4 development (lysis and the shutoff of macromolecular synthesis³) but the early events seem to be entirely normal (ref. 3 and H. Krisch, personal communication). In mixed infections at 42° C *tsG1* complements the late lysis phenotype of *amtA3* mutation and progeny phage are produced at the same time as in wild type infections (data not shown). As there are no other known genes in the gene 52 to *t* region, the *tsG1* mutation defines a new gene which is called *mot* for modifier of transcription.

SDS-polyacrylamide gel analysis of the proteins synthesised after infection of *Escherichia coli* B^E (*su*⁻) at 42° C with *amH17* or with *tsG1* is presented in Fig. 2. *amH17* was used as the control in this experiment because it maps close to *tsG1* and because it⁴ as well as *tsG1* has a DNA synthesis delayed phenotype. The initial rates of synthesis of the proteins as seen on these gels are identical in *amH17* and in wild-type infections (unpublished observations). Many differences between the *amH17* and *tsG1* infections can be seen. Since nearly the same amount of radioactivity precipitable by trichloroacetic acid was layered on each gel, differences in band intensities for individual proteins reflect differences in their relative rates of synthesis. In *amH17* infections the band corresponding to the *rIIA* protein is quite dense in the 2–6 min labelling, decreases in intensity during the 6–10 min labelling and is even less dense in the 10–14 min pulse. But in *tsG1* infections the *rIIA* band is at least as dense in the 6–10 min labelling as in the 2–6 min labelling and a considerable amount of it is still being synthesised in the 10–14 min pulse although its rate of synthesis is then slightly less than in the 6–10 min pulse. Other experiments show that in *tsG1* infections detectable amounts of *rIIA* protein are still synthesised as late as 35–40 min after infection at 42° C. Thus in *tsG1* infections the *rIIA* protein is synthesised for a considerably longer time that in either *amH17* or wild-type infections.

There are many mutants of T4 which affect the normal turnover of early gene functions: maturation-defective genes (33 and 55)^{5,6}; the DNA-delayed mutants⁷ and all of the DNA-negative mutants^{1,7}, have this property to varying extents although synthesis of the *rIIA* protein is not affected by any of these mutants to the extent that it is affected by *tsG1*. But *tsG1* is different in a much more important respect from all other mutants having an effect on early gene turnover. After infection with all these mutants the initial rates of synthesis are normal for all the early proteins which have been studied. Though most of the phage induced proteins do appear at the normal time in *tsG1* infected cells, the initial rates of synthesis for some of them are considerably reduced (see Fig. 2). The products of genes 43, 45 and *rIIB* are detectable during the first labelling period but their rates of synthesis are significantly reduced. P43 and P45 seem to be more severely affected than *rIIB*. Whereas most of the proteins from *tsG1* infections as seen on these gels appear at the normal time a few are not detectable during the first or even the second labelling period. For example, the band migrating at the position of the gene 32 protein is not visible until the 6–10 min labelling period.

The effect of *tsG1* on the expression of the *rIIB* protein is of special interest because it suggests an hypothesis for the mode of action of the *mot* gene product. Schmidt *et al.*⁸ have shown that the *rIIB* cistron is transcribed as part of a polycistronic message which includes the *rIIA* species and that it is also transcribed from a promoter located between the *rIIA* and *rIIB* cistrons. Preliminary evidence indicates that early after infection there is less *rIIB* transcription relative to *rIIA* transcription in *tsG1* infected cells than in cells infected with wild type. Since at early times in *tsG1*-infected cells there is a relative lack of *rIIB* message and protein but a normal amount of *rIIA* protein we hypothesise that in *tsG1* infected cells transcription from the

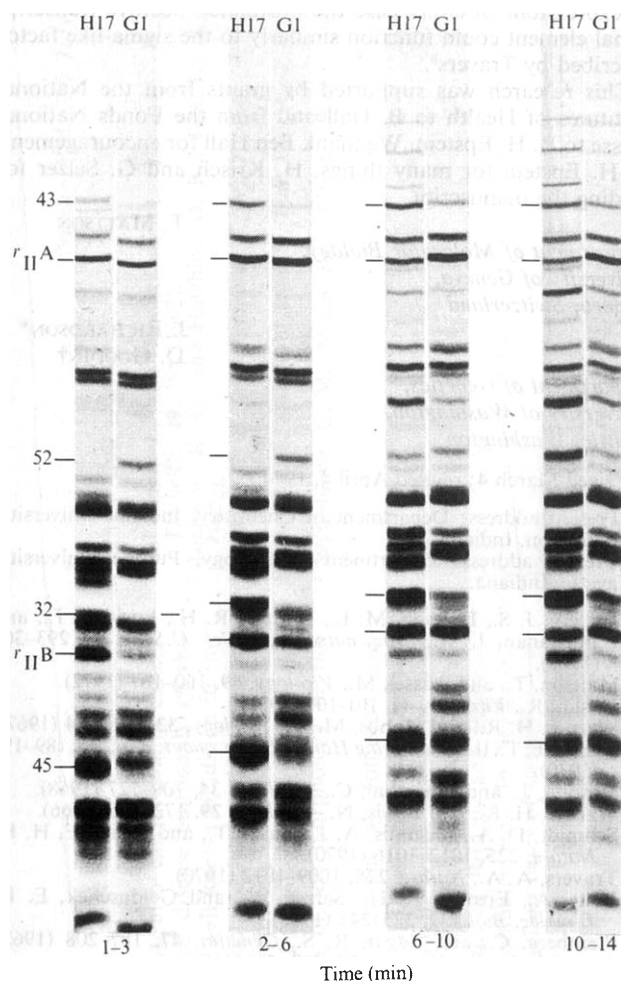


Fig. 2 SDS-polyacrylamide gel analysis. *E. coli* B^E (*su*⁻) grown in M9S containing 0.2% casamino acids to 10^8 ml⁻¹ was centrifuged and resuspended at 5×10^8 ml⁻¹ in M9S* (M9S containing only 10% of the normal amount of casamino acids). One millilitre of cells warmed for 2 min at 42° C were added to 24 ml of M9S* at 42° C containing either *tsG1* or *amH17* at 2×10^8 ml⁻¹. At 1, 2, 6, and 10 min after infection 5 ml aliquots of the infected cells were transferred to tubes at 42° C containing 5 μ Ci of a mixture of 15 ¹⁴C-labelled amino acids (NEN 445). The pulses 1–3, 2–6, 6–10 and 10–14 min were terminated by the addition of a 20-fold excess of unlabelled casamino acids. After a 3 min chase the samples were chilled by addition of ice and then centrifuged. The pellets were resuspended in 0.25 ml of 0.01 M Tris pH 7.5 and 1.0 ml of sample buffer (10% glycerol, 5% 2-mercaptoethanol, and 3% sodium dodecyl sulphate in 0.0625 M Tris pH 6.8) and then placed in boiling water for 3 min. Disc 10% SDS-polyacrylamide gels were prepared as described by Laemmli¹². The gels were run at a constant current of 1–2 mA per tube and were stained, sliced, dried and exposed to X-ray film as described by Fairbanks *et al.*¹³. About 30,000 trichloroacetic acid precipitable counts were layered on each gel. The numbers on the left margin of the figure identify the genes coding for the proteins indicated. The band migrating slightly faster than P32 is P44 (ref. 14). The absence of the gene 52 band in the early labellings with *amH17* is expected because *amH17* is in gene 52. The band appearing at the position of P52 in the last two labelling periods is a cleavage product of the main structural protein of the phage head. In the gels from the 1–3 min labelling, the migration of the proteins was not identical. The gels were matched in the P32–*rIIB* region and as a consequence the positions of P43 and *rIIA* in the gel from the *tsG1* infection are slightly lower than the corresponding bands in the gel from the *amH17* infection.

promoter located between the *rIIA* and *rIIB* cistrons is defective. Thus the normal *mot* gene product may have a positive role in transcription related to the recognition of promoters like the one between the *rIIA* and *rIIB* cistrons. The *mot* gene itself might code for this regulatory element or it may merely control

its expression. In either case the postulated positive transcriptional element could function similarly to the sigma-like factor described by Travers⁹.

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Immunotherapeutic suppression in transplantable solid tumours

α -FOETOPROTEIN (AFP) is a glycoprotein, of unknown function, synthesised by the foetal liver and yolk sac of mammals¹. Increased serum levels of AFP in adults are indicative of either pregnancy or a pathological state, that is primary hepatocellular carcinoma, regenerating liver and teratoblastomas of the ovary and testis^{2,3}. Recently, abnormal levels of AFP in amniotic fluids have been correlated with foetal distress⁴. While attempting to study the physiological role of AFP during ontogenic and oncogenic growth, we have observed an interaction between AFP and its homologous antiserum that may have immunotherapeutic value. We report here the suppression of mouse hepatoma growth *in vivo* and *in vitro* using passively administered antiserum to AFP.

Amniotic fluid (AF) containing AFP was obtained from C57BL mice as described by Sell⁵. AFP was isolated from the AF by upward flow gel filtration on Sephadex G-75, followed by preparative polyacrylamide gel (PAAG) column electrophoresis using a modification of Gussev's method⁶. A single band could be demonstrated subsequently by disc PAAG electrophoresis. The AFP isolate was emulsified with equal amounts of Freund's complete adjuvant for use as an immunogen. New Zealand white rabbits were immunised twice weekly for 2.5 weeks by subcutaneous flank injections. The rabbit antiserum, collected 2 weeks after the last injection was

immunoreactive against mouse AFP, but not albumin, as determined by microimmunodiffusion. A single band in the α_1 position was demonstrated after immunoelectrophoresis. The antiserum was absorbed further with nonimmune mouse serum (NMS) to insure monospecificity. Using the absorbed anti-AFP serum, AFP was quantitated in mouse sera using radial immunodiffusion (unpublished results of G. J. Mancini and S. R. Young).

Male and female mice of the C57L/J inbred strain bearing the AFP-secreting hepatoma BW7756 (Jackson Laboratory) were used as the animal tumour model. A standard tumour growth curve was obtained during 30 d (after transplantation) using mice weighing 20–28 g. The transplanted tumours usually exhibited 100% takes. The anti-AFP antisera were tested against the hepatoma during this period by three *in vivo* and two *in vitro* methods. The *in vivo* testing included (1) administration of graded doses during tumour growth; (2) a large, single dose administered 2 d after implantation; and (3) incubation of the tumour inocula in test serum before implantation. The *in vitro* methods encompassed (4) the cytotoxic antibody assay, and (5) tissue culture cytopathic testing. The tissue culture experiments, however, will be described in detail elsewhere⁷.

The graded doses included alternate daily intraperitoneal injections (a total of 2.60 ml) of absorbed anti-AFP serum given in small divided doses during 28 d. Using method (2), a single large dose of absorbed antiserum (2.0 ml) was injected intraperitoneally into the mice 2 d after transplantation. Method (3) was performed by incubating the tumour inocula (1.0 mm³ ml⁻¹ whole antiserum) for 30 min at 25°C immediately before implantation. The incubated tumour inocula were subsequently washed twice in Hanks balanced salt solution before implantation. All animals were observed and inspected daily for tumour growth over the post-transplantation period. At autopsy, 28 d later, all animals were bled and tumour weight and size were determined. For each experiment described above, nonimmune rabbit serum (NRS) absorbed with NMS was administered as control serum to an age-matched and weight-matched group of mice. The cytotoxic antibody assay was performed *in vitro* according to the method of Gorer and O'Gorman⁸. Fresh guinea pig serum was titred and used as a complement source. In some studies, only deactivated anti-AFP and NRS were used. For each determination, 3×10^6 – 4×10^6 screen-tested tumour cells were counted in a haemocytometer by trypan blue exclusion.

The *in vivo* methods of tumour growth suppression are compared in Table 1, which shows that antiserum administered gradually throughout incubation resulted in a 52% reduction in tumour size as compared with untreated controls (5% significance level). However, the NRS control group displayed a 20% reduction; thereby subtracting out to a final 32% reduction in the experimental group. Similarly, the animals

Table 1 The *in vivo* methods of immunotherapeutic tumour growth suppression are compared in C57L/J mice administered anti-AFP serum

Treatment groups	Mean	s.e.m.	Growth (%) reduction*	Significance level†
Graded dose (11)				
A·AFP	2.84	0.74	32.0	< 0.05
NRS	4.70	0.47		
Single dose (9)				
A·AFP	3.52	0.52	33.0	< 0.10
NRS	5.44	1.05		
Pre-incubated inocula (11)				
A·AFP	0.52	0.18	67.0	< 0.01
NRS	4.42	0.64		
Untreated (13)				
Control	5.87	0.50	0.0	—

The numbers of animals in the sample are given in parentheses.

* Treated control — Experimental
tumour weight — tumour weight

Untreated control
tumour weight

† Student's *t* test was used.

subjected to a single, large dose also underwent a 33% reduction in tumour growth even though the statistical significance of this latter experiment differed considerably from treatment with graded doses (10% compared with 5% probability level). Finally, treatment with incubated inocula demonstrated not only a high statistical significance (1% level) but also a 67% reduction in tumour weight. Thus, direct contact of the tumour cells in the test reagents before implantation resulted in cytotoxicity which was twice as effective as the other modes of treatment.

The results of the cytotoxic antibody assay *in vitro* are presented in Fig. 1. An innate cytotoxicity of nonimmune rabbit and guinea pig serum against mouse cells was observed which could be eliminated by a 1:10 to 1:16 serum dilution. Nonimmune rabbit serum control levels maintained a 70% tumour cell viability throughout a serum dilution range of 1:32 to 1:128. In contrast, the anti-AFP serum had a 100% cell killing effect at 1:16 and a 66% effect at 1:32 dilution. Unlike the NRS, which remained stable, the cytotoxicity of the AFP antiserum could be eliminated uniformly by extension to a 1:128 dilution as evidenced by the cytotoxic index in Fig. 1. Thus, the complete cell cytotoxicity ($CI = 1.0$) could be titred out over three two-fold dilutions. If the innate cell cytotoxicity of NRS for mouse cells is subtracted out (as above), it could still be observed that anti-AFP exerted a 30–33% killing effect on hepatoma tumour cells *in vitro* above that of NRS. Further cytotoxic testing using anti-AFP serum indicated that complement (host or foreign) was not an absolute requirement for cell killing but its presence intensified the killing effect. Finally, the cytotoxicity of the anti-AFP serum could be abrogated by absorption with purified AFP.

The gross pathology observed during the 30 d included fibroid accumulation, serous exudation, loss of connective tissue capsule and massive haemorrhaging at the tumour site. By 28 d, much of the tumour mass presented as nonviable tissue in mice treated with anti-AFP serum. In contrast, the mice administered NRS displayed only occasional haemorrhaging about the tumour mass. The corresponding histopathology revealed the presence of mononuclear infiltrates which invaded the tumour tissue of the animals treated with AFP antiserum. The cellular infiltrate populations increased uniformly from the 14th to the 21st day, and by day 28 much of the tumour mass had been replaced by inflammatory tissue. However, the animals treated with NRS displayed only focal infiltrates with occasional ischaemic necrosis. The histopathological results, which were confirmed by Dr J. J. McCoy jun. of the Veteran's Administration Hospital, Columbia, South Carolina, will be reported in more detail in a forthcoming publication.

The presence of AFP in serum, determined quantitatively by radial immunodiffusion, was suppressed in the antibody-treated mice, while those subjected to NRS exhibited a steady increase in AFP serum concentrations. The mice treated with NRS displayed a nine-fold increase in serum AFP from the 14th to the 28th day after transplantation (final AFP concentration = 9.75 mg ml^{-1}). In contrast, the mice treated with anti-AFP serum showed a slight increase in AFP production from the 14th to the 21st day with a drastic decrease thereafter to the 28th (final AFP concentration = 0.38 mg ml^{-1}). Thus, the presence of AFP in the serum seems to correlate well with tumour status.

Our results indicate that humoral immunotherapy was effective against a progressively growing solid tumour. The passive administration of heterologous antiserum to AFP in tumour-bearing mice resulted in a suppression of hepatoma growth and a decrease in AFP production over 30 d. The greatest reduction in tumour size (67%) occurred when the tumour inocula were incubated directly in the test serum. When the antiserum was administered by two methods of intraperitoneal injection, tumour size was reduced 32%. Extrinsic complement was not used or added to the serum; however, the serum was not heat deactivated and complement

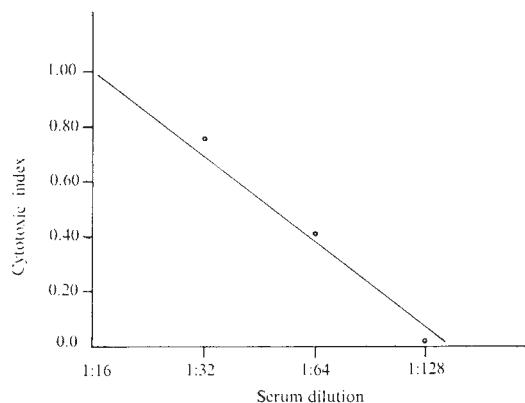


Fig. 1 Cytotoxic index for anti-AFP serum (exp) versus nonimmune rabbit serum (control) tested against mouse hepatoma target cells. Cytotoxic index is obtained from:

$$\frac{\text{Proportion of dead cells in control} - \text{Proportion of dead cells in exp}}{\text{Proportion of dead cells in control}}$$

may have been present in either the rabbit or mouse serum. In comparison with the *in vivo* test, the use of guinea pig complement *in vitro* also displayed a 30–33% cell cytotoxicity with direct incubation of teased tumour cells with various antibody dilutions. Neither native nor heterologous complement was required for *in vitro* cytotoxicity. Finally, the specificity of the cytotoxic reaction was demonstrated by absorption studies using purified antigen.

These experiments signify that some humoral factor or group of factors (anti-AFP) present in the sera of rabbits immunised to AFP induced growth retardation in progressively growing mouse hepatomas. One cannot rule out the possibility that soluble antigen-antibody complexes, present in the serum after absorption, were not involved in the observed suppression. Thus, the host defences may be responding to the presence of immune complexes in and about the tumour. In any case, the killing effect of the antiserum *in vivo* was probably due to (1) tumour cell-surface destruction, and (or) (2) vascular damage within and around the tumour mass. In either case, immune complexes would be formed which can act with or without complement. Since this immunotherapeutic approach is effective in tissue culture, the absolute requirement of active factors from the host mouse can be ruled out. The gross and histopathological evidence suggests that an Arthus-type tissue destruction occurred at the tumour site which, in effect, starved the tumour and retarded overall growth.

Since the function of AFP has not been established, it is unclear whether the actual blocking of AFP function played a role in the retardation of hepatoma growth. Some investigators have suggested that AFP functions in a growth factor capacity being essential for foetal liver and (or) hepatoma growth^{9,11}. Recently, indirect evidence has implicated AFP as a steroid (oestrogen) binding protein¹⁰. It is tempting to speculate that the tumour growth suppression reported here may have occurred at the level of hormonal induction of protein synthesis.

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Inhibition of cell-dependent cytotoxicity as an assay for mouse alloantibody

MANY assays for alloantibody depend on functional activities of the Fc portion of the immunoglobulin molecule. These functional activities are acquired when the antibody binds to its target cell. Complement binding, as demonstrated by lysis or complement fixation, has been the most widely used of these activities. In man, alloantibody can also be detected by its ability to mediate cell-dependent lysis of target cells¹⁻³. In this system, effector cells (human peripheral blood leukocytes) lyse allogeneic chromium ⁵¹Cr-labelled target cells (lymphocytes) in the presence of the appropriate alloantibody, providing a sensitive, complement-independent assay for alloantibody. In contrast, we have found that mouse alloantisera do not usually mediate lysis of mouse target cells (lymph node lymphocytes, phytohaemagglutinin (PHA) blast cells, and tumour cells) by mouse effector cells (spleen cells). Data supporting this conclusion will be presented elsewhere.

In man, alloantibody has a second activity in this cell-dependent assay: namely, that when the antibody is directed against the effector cell it inhibits cell-dependent killing of antibody-coated target cells². We have found that mouse alloantisera have a similar effector-cell inhibiting capacity. Mouse spleen cells are excellent effectors for lysing antibody-coated xenogeneic target cells (in contrast to their inability to lyse alloantibody-coated allogeneic cells). When alloantibody directed against H-2 specificities of the effector cell is added, the lysis is abolished. This assay, which we refer to as the cytotoxicity inhibition assay (CIA) is the subject of this report.

The cytotoxic system used is similar to that of Perlmann⁴, using mouse spleen cells (effector cells) to lyse chicken red cells (CRBC) (target cells) in the presence of rabbit anti-chicken red cell antibody (RACA). Single-cell suspensions of mouse spleen cells in Eagles' minimal essential medium (MEM) with 5% foetal calf serum were placed in plastic culture tubes. The alloantiserum to be tested was added to these, with 10⁴ radioactive ⁵¹Cr-labelled CRBC and RACA (1/25,000) in a final volume of 250 μ l. All experiments are performed in triplicate. Sheep red cells were added at 10⁷ ml⁻¹ to lower background ⁵¹Cr release⁵. The mixture was then incubated at 37° C for 3 h in a 10% oxygen, 5% carbon dioxide atmosphere in a dessicator. After 3 h, 500 μ l of cold MEM was added to each sample; the cells were spun down and the supernatant removed. Cells and supernatant were then counted separately on a Packard Autogamma scintillation spectrometer, and the results expressed as % ⁵¹Cr release.

Excellent ⁵¹Cr release with good sensitivity to inhibition by antibody occurred at spleen cell to CRBC ratios of 200:1 and RACA concentrations of 1/25,000, and these concentrations were used in all studies discussed here. Maximum killing usually ranged between 30% and 60% ⁵¹Cr release, with background ⁵¹Cr release of 0.5-3.0%. The alloantisera tested are conventional anti H-2 antisera, with good titres in complement

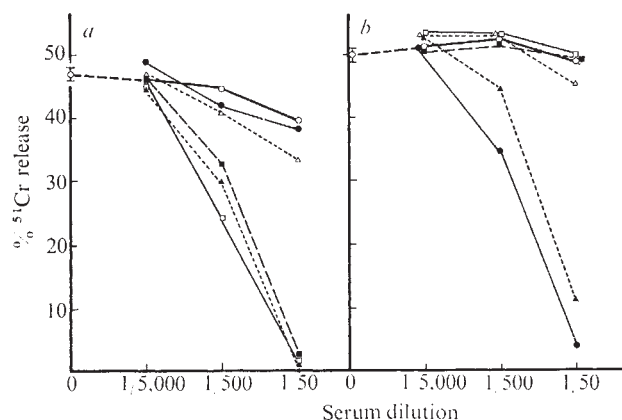


Fig. 1 The specificity of the CIA test. Various sera were incubated with BALB/c (H-2^d) or CBA/H (H-2^k) spleen cells (2×10^6) plus RACA (1/25,000) and CRBC (10^4) for 3 h. The reactions of the three anti H-2^d sera against BALB/c are very strong, whereas the anti H-2^k and anti H-2^b sera do not inhibit. The reaction of the anti H-2^k serum with CBA/H is similarly very strong. Serum 'b anti d', C57B16 anti BALB/c, reacts with CBA/H as would be predicted by public specificities shared by H-2^k and H-2^d. *a*, BALB/c effectors; *b*, CBA/H effectors. ○ 'NMS': Normal BALB/c serum; ● 'anti k': BALB/c anti CBA/H; △ 'anti b': (CBA/H × BALB/c) F, anti C57 B16; ▲ 'b anti d': C57B16 anti BALB/c; □ 'k anti d' No. 1: CBA/H anti P815Y; ■ 'k anti d' No. 2: CBA/H anti BALB/c.

mediated microcytotoxicity (up to 1/1000).

Many mouse alloantisera show a powerful specific inhibitory effect on cell-dependent antibody-mediated lysis of CRBC by spleen cells (Fig. 1). The effect of normal mouse serum, irrelevant antiserum or antiserum directed against specificities of the effector cell on ⁵¹Cr release are shown in Fig. 1. The killing by BALB/c spleen cells (H-2^d) is virtually eliminated by anti H-2^d antisera, but is only weakly inhibited by normal mouse serum, anti H-2^k serum, or anti H-2^b serum. Similarly, killing by CBA/H spleen cells (H-2^k) is strongly inhibited by anti H-2^k serum, but only weakly inhibited by anti H-2^d and anti H-2^b serum or by normal mouse serum. The exception, 'b anti d', anti H-2^d raised in C57B16 mice (H-2^d), reacts with CBA/H spleen cells in a manner predictable on the basis of public specificities shared between H-2^d and H-2^k (ref. 6). When allowance is made for this predictable cross-reaction, and when compared with the weak inhibitory activity of any mouse serum, the CIA is thus highly specific. The weak inhibitory activity of normal mouse serum and of irrelevant antiserum is similar to that noted in other systems⁷.

The specific inhibitory activity can be absorbed out (Fig. 2). Anti CBA/H serum which had been absorbed $1 \times$, $2 \times$, or $3 \times$ against 3×10^7 CBA/H spleen cells was assayed for its ability to inhibit killing by CBA/H spleen cells (Fig. 2*a*). One absorption produced a dramatic decline in the inhibitory activity, with further decline after each subsequent absorption. The cells used for absorption were resuspended and washed thoroughly and then assayed for their activity as effector cells (Fig. 2*b*). The first absorption leaves the cells used for this purpose with greatly reduced killing capacity, and each successive absorption reduces the killing capacity of the absorbing cells less. Thus, the inhibitory activity of the alloantiserum is absorbed out, and the absorbing cells become inhibited in their killing capacity. Additional experiments indicate that this inhibition of killing capacity lasts for at least 24 h when the antibody-coated effector cells are washed and incubated at 37° C.

We therefore feel that the CIA detects a specific, absorbable activity which is probably alloantibody in nature. We investigated whether this antibody is directed towards the H-2D and H-2K 'serologically-defined' specificities, or towards other H-2 or non H-2 antigens by testing an anti H-2^d serum and an anti H-2^k serum against various congenic mouse

strains, with a C57Bl/10 Sc Sn (B10) background, differing only in all or part of the H-2 region (Fig. 3). Here the specificity controls, BALB/c and CBA/H, show that the reaction is completely specific. The anti H-2^k serum causes strong inhibition of B10.BR, B10.AKM and B10.A, each of which shares part or all of its H-2 region specificities with H-2^k. Similarly, anti H-2^d serum inhibits killing by B10.A and B10.AKM cells which have part of their H-2 regions from H-2^d (B10.A) or from H-2^a (B10.AKM), which has many specificities in common with H-2^d. The weak reaction of both anti H-2^d and anti H-2^k sera with B10 cells is expected on the basis of shared public specificities.

While complete understanding of the specificities involved remains to be established, we believe that 'serologically-defined' H-2 specificities can explain most of the inhibitory activity of these alloantisera.

The mechanism of this inhibition is under study. The absence of complement from the medium, the high titres of inhibitory activity in the sera (1/3,500 or more) and cell counts made after many hours of incubation seem to make cell death or agglutination unlikely mechanisms. Damage to a small sub-population of cells, the actual effector cells, is impossible to rule out at present because these cells are morphologically unidentifiable. On the basis of current knowledge of the killing systems mediated by antibody and effector cells^{8,9}, the most likely mechanism is that 7S antibody attached to a surface antigen on either the effector cell or an adjacent cell, blocks the receptor by means of the Fc portion of the antibody, perhaps in the form of an immune complex. This would be in agreement with present concepts of the binding of immune complexes by the Fc receptor^{10,11}.

We cannot explain the dichotomy between the ability of mouse spleen cells to lyse antibody-coated CRBC and their apparent inability to lyse alloantibody-coated allogeneic cells. One report¹² documents the cell-dependent lysis of YAC and P815Y tumour cells coated with antibody raised against YAC tumour cells in CBA mice but the possibility that this is antitumour activity, and not anti H-2 activity, which is mediating this lysis has not been investigated*. If most alloantisera in mice cannot mediate cell-dependent target cell destruction, then what is the importance of the interaction of these antibodies

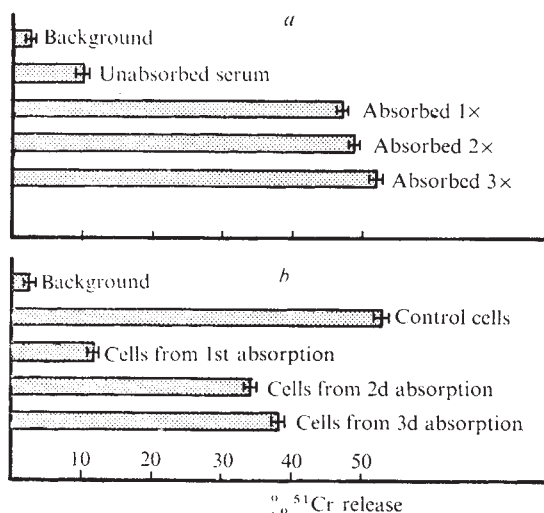


Fig. 2 The effect of absorption of serum with specific cells. 1 ml of BALB/c anti CBA/H (1/50) was absorbed for 30 min at room temperature with 3 x 10⁷ CBA/H spleen cells, once, twice, or three times. The inhibitory activity was then assayed against fresh CBA/H spleen cells at serum concentration 1/125. Similarly, the cells used for the absorption were resuspended and their cytotoxic activity was assayed to see if they had been inhibited. *a*, The inhibitory activity is greatly reduced after only one absorption; *b*, the cytotoxic activity of the cells used for the absorption is greatly reduced. All studies done for 3 h at 37° C at effector: CRBC ratios of 200:1 and RACA concentration 1/25,000. Absorption with BALB/c cells (not shown) has no effect.

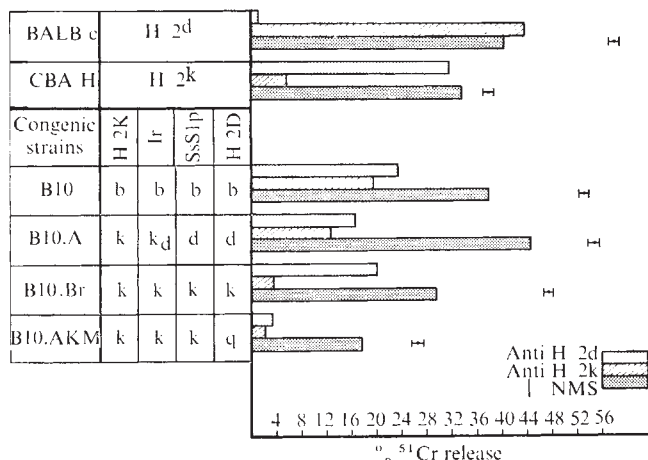


Fig. 3 Typical study of the association of inhibitory activity in alloantisera with H-2 antigens. Spleen cells (2 x 10⁶) were incubated with RACA (1/25,000) and chromium labelled CRBC (10⁵), in the presence of 1/50 normal mouse serum, anti H-2^d serum (CBA/H anti BALB/c) or anti H-2^k serum (BALB/c anti CBA/H). The controls with no added normal mouse serum are indicated (-●-). Complete specificity is shown on the basis of the reactions with BALB/c and CBA/H. The strong reactions of both sera with B10.A, and of anti H-2^k with B10.BR and B10.AKM are attributable to private and public specificities. The reactions of both sera with B10, and of anti H-2^d with B10.AKM, can be attributed to public specificities alone. The table indicates the source of the genetic material for this region in the H-2 complex. 'd', Derived from H-2^d; 'k', derived from H-2^k; 'b', derived from H-2^b; and 'q', derived from H-2^a (ref. 13).

with the Fc receptor of the effector cell? Perhaps the lack of killing of the target cell in such interaction in mice permits enhancement phenomena to occur more readily.

Finally, the CIA is negative with some sera that are weakly positive in complement-dependent lysis. This could be explained by differences in antibody classes: complement-dependent lysis will detect 7S and 19S antibody, whereas it seems likely the Fc receptor will be blocked only by 7S antibody. The sera which are positive in the CIA usually have titres higher than their complement-dependent cytotoxic titre. The CIA thus provides an important new alloantibody assay, independent of complement, for mouse immunogenetic studies, and also, as we shall report elsewhere, for immunogenetic studies in man.

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*Note added in proof: Similar arguments apply to other recent reports (Zigheboim, J., Bonavida, B., and Fahey, J. L., *J. Immunol.*, **111**, 1737; 1973; Britton, S., and Forman, J., *Transplantation*, **17**, 180; 1974).

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Inhibition and reversal of capping by cytochalasin B, vinblastine and colchicine

THE phenomenon of capping in lymphocytes¹⁻⁴ consists of the segregation of membrane components, specifically cross-linked by antibody or other ligand, at one pole of the cell, typically that surrounding the centrosome^{1,2}. Capping has been interpreted as resulting from a countercurrent flow of membrane components, in which the cross-linked patches are transported towards the centrosome region by interaction with cytoplasmic structures, while the unaffected parts of the membrane flow in the opposite direction^{1,2,5}.

This interpretation indicated a possible role in capping of contractile microfilaments and microtubules. With the exception of a negative report⁶, cytochalasin B, which apparently impairs microfilament function⁷, was found to inhibit consistently, although in most cases only partially, capping of surface immunoglobulin (Ig) (refs 1, 3, and 8). Microtubule-affecting drugs, like colcemid and vinblastine, had no inhibitory effect^{1,8}. It has been suggested, however, that microtubules could be involved in the control of surface Ig movement, as the inhibition by con A of capping of surface Ig-anti-Ig complexes could be reversed by high doses (10⁻⁴ M) of vinblastine and colchicine^{8,9}. By studying the combined effect of vinblastine or colchicine and cytochalasin B on capping of surface Ig and con A receptors on mouse spleen lymphocytes, I have now found evidence indicating that both microfilaments and microtubules can play a direct role in capping.

Rhodamine-labelled rabbit anti-mouse immunoglobulin (rh-RaMIg), fluorescein-labelled sheep anti-mouse immunoglobulin (fl-SaMIg) and rhodamine-labelled rabbit anti-sheep immunoglobulin (rh-RaSIg) were prepared according to Cebra and Goldstein¹⁰. Con A (Miles-Yeda) similarly was conjugated to fluorescein¹⁰ (fl-con A) in the presence of 0.1 M glucose, 5 × 10⁻⁴ M MnCl₂ and 5 × 10⁻⁴ M CaCl₂ and purified by chromatography on Sephadex G-50. Only that fraction which strongly bound to Sephadex at pH 7.2., that is, which in a stepwise elution could be eluted between 0.01 M and 0.10 M glucose, was used. BALB/c spleen cells were incubated at 37° C in Leibovitz medium (L15) (Flow) containing 0.2% bovine serum albumin. This medium does not contain glucose, reducing the possibility of secondary effects due to inhibition of glucose transport by cytochalasin^{11,12}.

Vinblastine (5 × 10⁻⁵ M or less) had no effect on, or slightly enhanced the percentage of cells capped by rh-RaMIg, while cytochalasin B (10 or 20 µg ml⁻¹) was partially inhibitory (Table 1). However, when cytochalasin B and vinblastine were used together, capping was virtually completely inhibited (Table 1), even after several hours. The same effect was obtained substituting colchicine for vinblastine (Table 2). The inhibition occurred with these drugs in a dose range of 10⁻⁴ – 10⁻⁶ M, at which they are known to affect microtubules^{13,14}. The antibody was distributed as random spots all over the inhibited round cells (Fig. 1a). Using a second label (rh-RaSIg) to stain the first fluorescent antibody (fl-SaMIg), it was found that several, but not all, of these spots were intracellular. The combined use

Table 1 Capping of surface Ig in the presence of vinblastine and cytochalasin B

Sample	% caps
Experiment 1	
1 Control	94
2 Vinblastine 2.5 × 10 ⁻⁵ M	92
3 Cytochalasin B 10 µg ml ⁻¹	66*
Experiment 2	
Cytochalasin B (18 µg ml ⁻¹) present in all samples:	
4 No vinblastine	84†
5 Vinblastine, 5 × 10 ⁻⁵ M	8
6 Vinblastine, 1 × 10 ⁻⁵ M	8
7 Vinblastine, 5 × 10 ⁻⁶ M	13
8 Vinblastine, 1 × 10 ⁻⁶ M	18
Recovery (≤ 0.1 µg ml ⁻¹ cytochalasin B):	
5a Recovery from 5 (vinblastine, 5 × 10 ⁻⁵ M)	27†
6a Recovery from 6 (vinblastine, 1 × 10 ⁻⁵ M)	78†
7a Recovery from 7 (vinblastine, 5 × 10 ⁻⁶ M)	88†

Washed BALB/c spleen cells (2 × 10⁷ cell ml⁻¹) filtered through glass wool, were preincubated 35 min (Experiment 1) or 90 min (Experiment 2) at 37° C in vinblastine or cytochalasin B as indicated. Each sample (20–40 µl) was incubated with 20–40 µl of rh-RaMIg, 15 min at 37° C and washed once by centrifugation (always in the presence of the drugs). Part of samples 5, 6, 7 were washed with medium containing vinblastine, but not cytochalasin B, and incubated for 15 to 30 min at 37° C, before examination. Samples with cytochalasin B in this and other experiments contained 0.25% dimethylsulphoxide which had no effect on capping. Cells were scored as 'caps' if the stain was detectable over no more than one half of the cell, irrespective of whether it was on the surface or pinocytosed.

* In other experiments the inhibition varied from 20 to 70%.

† Mainly pinocytosed label.

of cytochalasin and vinblastine therefore also blocked the polar accumulation of this pinocytosed material, which was not completely prevented by either drug alone. The cooperative inhibitory effect of these drugs on capping was even more apparent when the inhibited cells were resuspended in fresh medium containing vinblastine or colchicine, but no cytochalasin (< 0.2 µg ml⁻¹, final concentration). The inhibition was reversed (Tables 1 and 2) and in less than 15 min all the label accumulated at one pole of the cell. Most of the label seemed, however, to be intracellular, that is, pinocytosed. The recovery was essentially complete in the presence of 10⁻⁴ M colchicine or 10⁻⁵ M vinblastine, incomplete at higher doses of vinblastine (Tables 1 and 2). If colchicine was removed and cytochalasin left in the medium, the recovery was incomplete (Table 2).

Cytochalasin B was even more effective on capping of con A receptors by fl-con A. At doses of 20–100 µg ml⁻¹ this fl-con A

Table 2 Inhibition of capping of surface Ig by cytochalasin B and colchicine

Sample	% caps
1 Control	85
2 Colchicine (10 ⁻⁴ M)	98
3 Cytochalasin B 20 µg ml ⁻¹	71
4 Cytochalasin B (20 µg ml ⁻¹) + 10 ⁻⁴ M colchicine	5
5 Cytochalasin B (20 µg ml ⁻¹) + 10 ⁻⁵ M colchicine	8
6 Cytochalasin B (20 µg ml ⁻¹) + 10 ⁻⁶ M colchicine	17
Recovery (< 0.2 µg ml ⁻¹ cytochalasin B):	
4a Recovery from 4 (colchicine, 10 ⁻⁴ M)	83
5a Recovery from 5 (colchicine, 10 ⁻⁵ M)	82
Recovery (15 µg ml ⁻¹ cytochalasin B):	
4b Recovery from 4 (colchicine, < 10 ⁻⁸ M)	30
5b Recovery from 5 (colchicine, < 10 ⁻⁹ M)	36

4 × 10⁷ cells ml⁻¹ were preincubated at 37° C for 75 min in the presence of colchicine and cytochalasin B, as indicated, and then incubated with rh-RaMIg for 15 min at 37° C. Part of samples 4 and 5 were washed to remove either colchicine or cytochalasin B from the samples, and incubated for 15 min at 37° C (recovery).

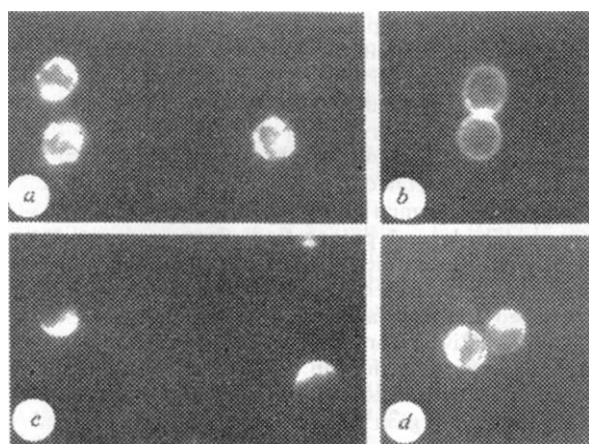


Fig. 1 Inhibition and reversion of caps. The photographs were taken using a Leitz Ortoplan microscope equipped with Opak-Fluor vertical illumination (Leitz GmbH-Wetzlar). *a*, Cells stained with rh-RaMIg at 37° C in the presence of 20 $\mu\text{g ml}^{-1}$ cytochalasin B and 10^{-6} M colchicine (see Table 2); *b*, cells stained with 50 $\mu\text{g ml}^{-1}$ of fl-con A at 37° C in the presence of 20 $\mu\text{g ml}^{-1}$ cytochalasin B; *c*, cells stained for 10 min at 23° C with rh-RaMIg in the presence of 5×10^{-5} M vinblastine. Typical caps; *d*, the same, but incubated further for 35 min at 37° C with 20 $\mu\text{g ml}^{-1}$ cytochalasin B. Spotty rings, with some accumulation of label on one side of the cell.

preparation inhibited surface Ig capping^{3,8} only by 30–40% and formed con A caps in about 50% of the spleen cells in 10 min at 37° C. This capping was completely and reversibly inhibited by 10 min exposure to 10 $\mu\text{g ml}^{-1}$ of cytochalasin B, either in the presence or in the absence of vinblastine (Table 3; Fig. 1*b*). The change in cell shape from round to variably elongated which accompanied con A capping was also inhibited by cytochalasin.

Table 3 Inhibition of capping of con A receptors by cytochalasin B and vinblastine

Sample	% caps
Experiment 1	
1 Control	32
2 Vinblastine, 10^{-4} M	51
3 Cytochalasin B (10 $\mu\text{g ml}^{-1}$)	<1
4 Vinblastine, 10^{-4} M + cytochalasin B (10 $\mu\text{g ml}^{-1}$)	0
Recovery (<0.15 $\mu\text{g ml}^{-1}$ cytochalasin B):	
3a Recovery from 3 (no vinblastine)	45
4a Recovery from 4 (vinblastine, 10^{-4} M)	36
Experiment 2	
5 Vinblastine, 10^{-5} M + cytochalasin B (10 $\mu\text{g ml}^{-1}$)	0
5a Recovery (cytochalasin B <0.1 $\mu\text{g ml}^{-1}$, vinblastine, 10^{-5} M)	52
5b Recovery (cytochalasin B 10 $\mu\text{g ml}^{-1}$, vinblastine $\leq 5 \times 10^{-8}$ M)	0

4×10^7 spleen cells were preincubated for 30 or 60 min at 37° C in vinblastine or cytochalasin B or in medium alone, then 90 μl of each sample were incubated with 10 μl of fl-con A (50 $\mu\text{g ml}^{-1}$ final concentration) for 15 min at 37° C. Part of samples 3, 4 and 5 were washed with medium free of cytochalasin or, respectively, vinblastine, as indicated, and incubated for further 30 min at 23° C (samples 3a, 4a) or 20 min at 37° C (samples 5a, b) before examination.

These findings demonstrate that cytochalasin-sensitive structures play an essential role in cap formation, and that microtubules are also somehow involved. The nature of the interaction between these structures is unknown. It is likely that the former corresponds to a cortical layer of microfilaments which interact directly with membrane elements⁷. The latter may interact indirectly with the membrane, perhaps through the mediation of microfilaments, and probably constitutes at least part of the internal cellular frame with respect to which mem-

brane movement occurs^{2,5}. As capping is not blocked by microtubule disruption, it seems likely that part of this frame and its polarity are preserved by other cellular structures. The different degree of inhibition by cytochalasin B on con A receptors and surface Ig capping could reflect differences in their interaction with cytoplasmic structures. It is also conceivable, however, that the function of these structures is affected only incompletely by cytochalasin B, to an extent sufficient to block cell movement, but not partial membrane displacements, which can occur also in the absence of cell movement^{2,5}. Differences in the extent of segregation achieved could then be quantitative and depend on the degree of cross-linking, size and number of the patches formed by a particular ligand.

Further evidence for the role of cytochalasin-sensitive structures, which indicates that they are involved in holding together the capped membrane, was provided by experiments in which preformed caps were 'reversed' into rings using cytochalasin. Cytochalasin B (10 $\mu\text{g ml}^{-1}$), either in the presence or the absence of vinblastine, reversed almost 90% of con A caps in 45 min at 37° C (Table 4), whereas little or no reversion was observed in rh-RaMIg caps. A large part of rh-RaMIg, however, was already pinocytosed within 10 min before the addition of cytochalasin, whereas, on the contrary, most of the capped con A remained on the surface. Repeating the experiment with

Table 4 Reversal of caps by cytochalasin B

	Time (min)	% caps
Experiment 1		
fl-con A caps (37° C, no vinblastine)	0	45
	45	6
Experiment 2		
rh-RaMIg caps (23° C, 5×10^{-5} M vinblastine)	0	91
	35	34

In Experiment 1, fl-con A caps were induced in 10 min at 37° C as described in Table 3 ($t=0$), then 10 $\mu\text{g ml}^{-1}$ of cytochalasin B were added and incubation continued, without washing, for 45 min at 37° C. In Experiment 2, spleen cells, preincubated for 2 h at 37° C with 5×10^{-5} M vinblastine, were incubated with rh-RaMIg at 23° C for 20 min. After washing, part of the sample was examined ($t=0$) and the rest incubated for 35 min at 23° C in the presence of 20 $\mu\text{g ml}^{-1}$ of cytochalasin B.

rh-RaMIg at 23° C and in the presence of vinblastine, which favours the formation of a true surface cap before the onset of appreciable pinocytosis, rh-RaMIg caps could be reversed by cytochalasin into spotty rings in about 60% of the cells (Fig. 1*b, c*; Table 4). Other (unpublished) data indicate that con A 'reversions' can also be induced by metabolic inhibitors, in agreement with an earlier report of Sällström and Alm¹⁵. These findings indicate that cytochalasin-sensitive structures are responsible for holding together the labelled patches into a single cap, even when these are not completely cross-linked by the ligand into a single complex; microtubules seem to be less important. On adding cytochalasin, the labelled patches are released and can mix again with the unlabelled membrane.

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Inhibition of surface capping of macromolecules by local anaesthetics and tranquillisers

IMMUNOGLOBULIN (Ig) molecules on the surface of B lymphocytes aggregate into a 'cap' when the cell is treated with anti-Ig¹⁻³. A similar process occurs with concanavalin A (con A) on neutrophil polymorphonuclear leukocytes (PMN)⁴. Capping occurs in two stages in both of these cell types^{4,5}: first, the ligand-receptor complexes aggregate into multiple clusters of variable size, leaving intervening bare membrane; and second, the clusters are swept into a single mass. Both stages are temperature-dependent (that is, they do not occur in the cold), but only the latter is energy-dependent (that is, clustering occurs but capping is inhibited on cells treated with metabolic inhibitors)⁴⁻⁶. The formation of clusters can be attributed to the ability of the ligand to cross-link receptors⁵; some form of membrane activity then sweeps the clusters into the cap. Capping can occur in the absence of cell locomotion^{4,7}, but if locomotion does occur the cap is found at the trailing end of the cell⁴. These aspects of capping have recently been analysed in detail^{4,7}.

We have now found that capping of con A on PMN, and of anti-Ig-Ig complexes on lymphocytes, is reversibly inhibited by local anaesthetics and tranquillisers which act as membrane stabilisers, characteristically able to inhibit membrane depolarisation⁸⁻¹¹.

Human PMN attached to glass coverslips⁴ were exposed to fluorescein-conjugated con A (100 $\mu\text{g ml}^{-1}$) in Hanks balanced salt solution (BSS) for 10 min at 4° C, washed in BSS, incubated (in the presence or absence of drugs) in BSS containing 10% foetal calf serum for 10 min at 4° C and then warmed to 37° C. In agreement with our previous observations⁴, fluorescence microscopy showed that, in the absence of drugs, approximately 95% of such cells developed caps of con A within 5 min; the PMN were motile and the caps were situated at the trailing end of the cell. Capping was inhibited in the presence of the local anaesthetics and tranquillisers; in addition, cell locomotion was suppressed. Testing with a range of doses (from 10^{-6} M to 4×10^{-2} M) established that complete inhibition of capping and locomotion occurred with 4×10^{-2} M Xylocaine, 10^{-3} M Nupercaine, 2×10^{-4} M chlorpromazine and 10^{-4} M trifluoperazine. Reversibility of this phenomenon was tested by washing away the drug just before warming to 37° C: capping was restored on approximately 95% of cells after treatment with Xylocaine, on 85% after Nupercaine, on 75% after chlorpromazine and on 50% after trifluoperazine.

The effect of Xylocaine on the redistribution of con A receptors on PMN was examined by electron microscopy of

shadow-cast surface replicas (using *Busycon canaliculatum* haemocyanin as a marker for con A¹²). Glass-attached PMN were exposed to con A (100 $\mu\text{g ml}^{-1}$) in BSS for 10 min at 4° C, washed in BSS, treated with haemocyanin (500 $\mu\text{g ml}^{-1}$) in BSS for 10 min at 4° C, washed in BSS and incubated (in the presence or absence of Xylocaine) in BSS containing 10% foetal calf serum for 10 min at 4° C. The cells were then warmed to 37° C for 10 min before fixation in glutaraldehyde and preparation of surface replicas⁴. As previously described⁴, electron microscopy of such specimens showed a random, dispersed distribution of con A on control cells labelled and fixed at 4° C; after warming to 37° C, the cells showed active locomotion and the con A quickly formed a single aggregate at the tail; if cell motility was impaired (for example, with cytochalasin B) capping occurred, but over the central region of the cell⁴. On PMN treated with Xylocaine, the con A aggregated into multiple, irregular, scattered patches at 37° C, but did not cap. Thus, capping was inhibited but not the temperature-dependent formation of patches.

We then studied the effect of these drugs on the capping of anti-Ig on lymphocytes. Mouse spleen cells were incubated with fluorescein-conjugated rabbit anti-mouse Ig (RAMG)⁵ for 15, 30 or 45 min at room temperature in the presence or absence of the drugs. After fixation in paraformaldehyde, the pattern of labelling was assessed by fluorescence microscopy. Capping was inhibited to different degrees by each drug (using the doses, mentioned above, which caused complete inhibition of con A capping on PMN) (Fig. 1). The inhibition by Xylocaine was complete. On the uncapped cells, label was distributed in multiple patches over the surface; such patches were small, in contrast to the large aggregates seen on metabolically inhibited lymphocytes⁵. Reversibility of this drug inhibition was tested in two ways: cells were exposed to the drug for 30 min, washed, and treated with RAMG for 30 min without the drug; or cells were treated with RAMG in the presence of the drug for 30 min, washed and incubated without RAMG or the drug for 30 min. Figure 2 shows that removal of the drug restored capping ability. This reversibility was particularly striking with Xylocaine.

Local anaesthetics inhibit membrane depolarisation by reducing sodium and potassium conductances⁹. This seems to follow displacement of calcium ions from binding sites in the membrane^{9,11,13,14}. In erythrocytes, such treatment leads

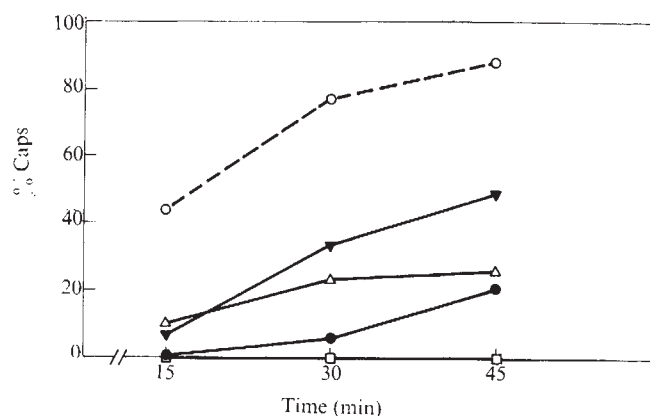


Fig. 1 Lymphocytes were collected from mouse spleens and purified by centrifuging on a Ficoll-Hypaque gradient; 5×10^6 cells (in 0.5 ml of Hanks BSS containing 1% foetal calf serum and the appropriate drug) were placed on tissue culture dishes; 50 μg of fluorescent RAMG was added and the suspension was incubated for 15, 30 or 45 min at room temperature, at which time an equal volume of 2.5% paraformaldehyde was added to stop the reaction. O, Control; □, 4×10^{-2} M Xylocaine; ▼, 10^{-3} M Nupercaine; ●, 2×10^{-4} M chlorpromazine; △, 10^{-4} M trifluoperazine.

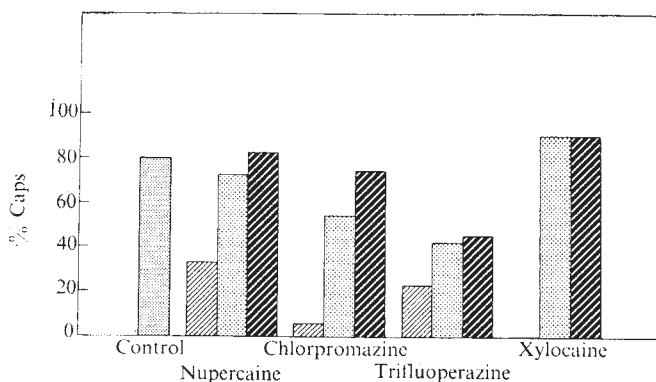


Fig. 2 Reversibility of the inhibition produced by the drugs. Light hatching, Lymphocytes (5×10^6 in 0.5 ml) were incubated with the drug for 30 min in the presence of 50 μ g of fluorescent RAMG. (Note complete inhibition by Xylocaine). Coarse dots, Lymphocytes were incubated with the drug for 30 min, washed once and then incubated in fresh media containing RAMG for 30 min. Heavy hatching, Lymphocytes were incubated with the drug and RAMG for 30 min, washed once, and then resuspended in media free of drug and RAMG for 30 min.

to expansion^{15,16} and physical stabilisation¹⁰ of the membrane, providing protection against osmotic lysis¹⁷. Other effects of such drugs include the inhibition of several kinds of cell-to-cell interaction (platelet adhesiveness^{18,19}, cell fusion²⁰, leukocytic adherence to endothelium²¹), as well as inhibition of phytohaemagglutinin-induced lymphocytic transformation²². Our experiments indicate that they also affect the redistribution of membrane-bound ligands. Whether this is due to 'molecular packing' induced by the burying of drug molecules within the membrane⁹, or distortion and expansion of membrane protein¹⁵, or some other intrinsic membrane derangement is not clear. Because the movement of patches into caps may depend on cytoplasmic influences (such as an intact metabolism, and a colchicine-sensitive system and (or) cell locomotion)⁴⁻⁷, another possibility is that the drugs affect cytoplasmic constituents. Thus, the immobilisation of PMN during drug exposure (also reported for procaine-treated fibroblasts²³) may be due to an inhibition of release of internal calcium causing an inability to activate the contractile process²⁴. In addition, Xylocaine has been reported to cause a reversible disappearance of microtubules in rabbit vagus nerve²⁵. This may have relevance to recent data from this laboratory showing that capping can occur with a cross-linking ligand in the absence of cell movement, but only if a colchicine-sensitive system is intact^{4,7}.

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Increased sensitivity of cell-free protein synthesis to double-stranded RNA after interferon treatment

NATURALLY occurring and synthetic double-stranded RNAs (dsRNAs) are interferon inducers¹. They inhibit protein synthesis in animal cells² and cell-free systems³⁻⁵. It is intriguing, therefore, that interferon treatment renders cells more sensitive to the toxic effects of dsRNA⁶, particularly as viral dsRNA may be involved in the replication of RNA viruses and has been isolated from DNA virus-infected cells⁷. Accordingly, the production of viral dsRNA in response to infection could bring about a general inhibition of protein synthesis in the interferon-treated cell, as is indeed observed in interferon-treated, vaccinia virus-infected mouse fibroblast L cells^{8,9}. The cell dies but little virus is produced: an effective way of limiting a natural infection.

Here we report an interferon-induced increase in the sensitivity of virus protein synthesis to inhibition by dsRNA in cell-free systems from interferon-treated L cells. Previously, we showed a similar enhanced inhibition of protein synthesis in such systems in response to infection with vaccinia or encephalomyocarditis (EMC) virus^{10,11}. The possibility that, in accord with the above hypothesis, this enhanced interferon-mediated inhibition of protein synthesis seen after infection is triggered by the synthesis of very small amounts of viral dsRNA is discussed.

The cell-free extracts used throughout were crude post-mitochondrial supernatant fractions from uninfected L cells. They were neither preincubated to lower endogenous incorporation nor passed through Sephadex to reduce the pool of cold amino acids. With such systems, a two to five-fold stimulation of amino acid incorporation in response to added EMC RNA as message is routinely observed and this stimulation is normally only slightly inhibited ($\leq 30\%$) by treatment of the cells with low doses (≤ 300 reference or 30 effective units ml^{-1} , see legend to Fig. 1) of interferon¹⁰. It is known that the added EMC RNA is translated correctly in these systems to yield EMC-specific polypeptides, initiation occurring at a single major site as in the intact EMC-infected cell¹²⁻¹⁶.

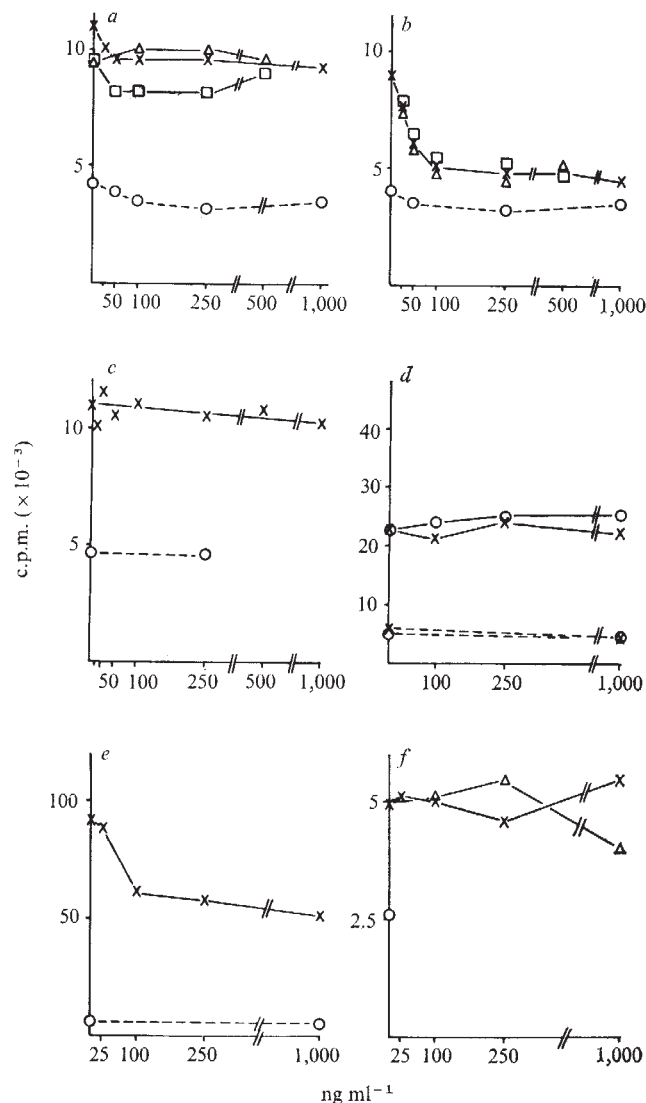


Fig. 1 Sensitivity of various cell-free systems to dsRNA and DNA. Crude postmitochondrial supernatant fractions were used throughout. The extracts in *a*, *b*, *c*, *d* and *f* were from L cells, that in *e* from Krebs cells. Interferon pretreatment of the cells was with 200 (*b*) or 300 (*d* and *f*) units ml^{-1} . The interferon concentrations throughout are in International Reference units based on the NIH standard. Ten reference units were equivalent to approximately one effective unit in reducing the yield of EMC by 50%. The heat-inactivated interferon (*c*) was at a concentration equivalent to 300 units ml^{-1} before inactivation; the heat treatment (60°C for 20 min) was just sufficient to destroy its antiviral activity. *a*, Control; *b*, interferon; endogenous amino acid incorporation $\pm$ increasing amounts of reovirus dsRNA ($\times$) and *P. chrysogenum* ($\square$) dsRNAs or poly(I)·poly(C) (Δ). The abscissa in all cases gives the range of final concentrations of the dsRNA, poly(I)·poly(C), or (in *f*) DNA in the assay. *c*, Heat-inactivated interferon; endogenous ($\circ$) and EMC RNA-stimulated ($\times$) amino acid incorporation $\pm$ reovirus dsRNA. *d*, Endogenous (---) and poly U-stimulated (—) ^3H -phenylalanine incorporation $\pm$ poly(I)·poly(C) with extracts from interferon-treated ($\times$) and control ($\circ$) cells. *e*, Krebs cell-free system; endogenous ($\circ$) and EMC RNA-stimulated incorporation ($\times$) $\pm$ reovirus dsRNA. *f*, Endogenous ($\circ$) and EMC RNA-stimulated incorporation with extracts from interferon-treated L cells $\pm$ calf thymus (Δ) and adenovirus ($\times$) DNA. The interferon pretreatment of the cells, the preparation of the cell-free extracts and their assay $\pm$ EMC RNA or poly(U) have already been described¹³. The ordinates give the ^{14}C -amino acid (mixture) or ^3H -phenylalanine incorporation (in c.p.m. $\times 10^{-3}$ per 50 μl assay)¹³. Mouse L cell interferon purified by affinity chromatography²¹ ($\geq 5 \times 10^7$ units per mg protein) from Dr K. Paucker was used in most experiments. Electrophoretically purified interferon ($> 10^7$ units per mg protein) was from

MRE Porton, England. To check the effectiveness of the interferon treatment, samples of the cells used to prepare the extracts in these experiments were plated as monolayers, infected with 4–10 plaque-forming units per cell of EMC and the virus yield after 17 h at 37°C was assayed by haemagglutination. Reovirus dsRNA was from Dr J. J. Skehel, *P. chrysogenum* virus dsRNA²² from Dr R. A. Cox and adenovirus DNA from Dr W. C. Russell. Poly(I)·poly(C) lot 8, Control No. 11–8–321 and poly(U) were from Miles-Seravac (Pty) Ltd, Maidenhead, Berkshire, England.

In such cell-free systems from interferon-treated L cells the translation of added EMC RNA was markedly inhibited (50–90%) by reovirus and *Penicillium chrysogenum* phage dsRNAs and by poly(I)·poly(C) (Fig. 1*b*). These dsRNAs had much less effect on the corresponding untreated systems (Fig. 1*a*), on endogenous incorporation (Fig. 1*a* and *b*) or on the translation of poly(U) (Fig. 1*d*). The insensitivity of the untreated L cell system could be analogous to that of the chick fibroblast cell-free system¹⁷, but is in contrast to the rabbit reticulocyte and, to a lesser extent, the Krebs cell-free systems (Fig. 1*e*) (refs 3–5). The basis for this is not known.

On occasion, particularly on pretreatment of L cells with higher concentrations of interferon, we have observed some inhibition of the translation of EMC RNA in the cell-free system in the absence of infection or dsRNA. Such systems, however, still showed an enhanced sensitivity to dsRNA. The addition of dsRNA to a 50% inhibited system from cells pretreated with 500 reference units ml^{-1} of interferon for example, resulted in a virtually complete inhibition of the translation of the added EMC RNA.

The minimum concentration of dsRNA required for inhibition in cell-free systems from interferon-treated L cells ($10\text{--}50 \text{ ng ml}^{-1}$, Fig. 1*b*) was higher than that required in the reticulocyte cell-free system ($0.1\text{--}1 \text{ ng ml}^{-1}$) but was of the same order as that observed for the Krebs system⁴ (Fig. 1*e*). No inhibition of incorporation was observed with similar concentrations ($10\text{--}1,000 \text{ ng ml}^{-1}$) of adenovirus, calf thymus or SV40 DNA, rRNA, poly(I), poly(C) or alkali-digested poly(I)·poly(C) all of which gave data essentially identical to those shown in Fig. 1*f*. These results indicate that the inhibitory agent is indeed dsRNA and that the major inhibitory effect is likely to be on the initiation of polypeptide chains^{3–5} rather than on chain elongation as represented by the majority of endogenous incorporation in these systems. The rate and extent of EMC RNA-stimulated incorporation were both inhibited by poly(I)·poly(C) in cell-free systems from interferon-treated L cells (Fig. 2).

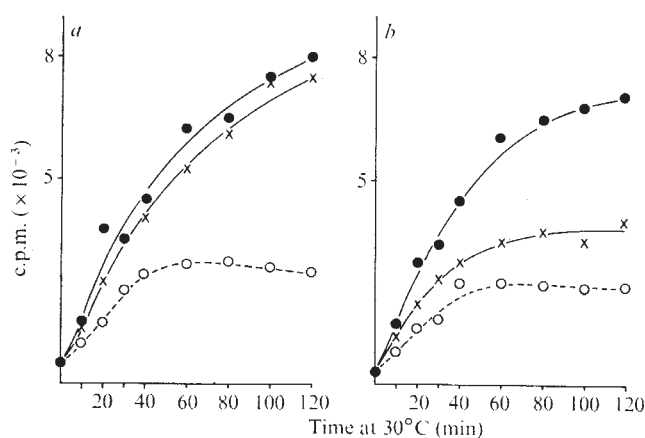


Fig. 2 Time course of amino acid incorporation in L cell-free systems from *a*, Control and *b*, interferon-treated (30 reference units ml^{-1}) cells. Endogenous ($\circ$) and EMC RNA-stimulated ^{35}S -methionine incorporation in the presence ($\times$) and absence ($\bullet$) of poly(I)·poly(C) (250 ng ml^{-1}).

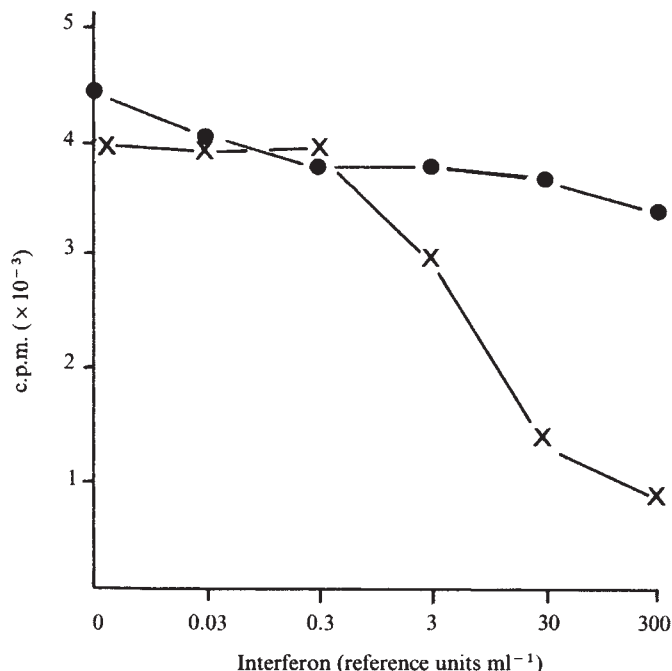


Fig. 3 Dependence on interferon concentration of the enhanced sensitivity to dsRNA of EMC RNA-programmed cell-free protein synthesis. Ordinate: EMC RNA-stimulated amino acid incorporation in the L cell-free system in the absence (●) and presence (x) of 250 ng ml⁻¹ of poly(I):poly(C). Abscissa: interferon concentration used in treatment of the L cells¹³ before isolation of the cell-free extracts. Ten reference units (legend Fig. 1) of interferon were equivalent to approximately 1 effective unit. Accordingly, here EMC virus growth was inhibited by 30, ≥97 and ≥97% when assayed (legend Fig. 1) in samples of these cells exposed to 3, 30 and 300 reference (0.3, 3 and 30 effective) units ml⁻¹, respectively, of interferon.

The inhibition was relatively independent of extract concentration and was observed at all concentrations of EMC RNA tested.

An important consequence of these results is that in any experiment involving a comparison of the translation of different viral and host mRNAs in cell-free systems from interferon-treated cells, it will be necessary to consider the possible effect of even very small amounts of intrinsic or co-tamining dsRNA structures.

The enhanced sensitivity to dsRNA has been seen with many cell-free extracts from eight different batches of L cells exposed to 10–500 reference (1–50 effective) units ml⁻¹ of four different preparations of highly-purified mouse interferon (>10⁷ reference units per mg protein) from two different laboratories. A dose response curve is shown in Fig. 3. Cell-free systems from L cells pretreated with interferon inactivated with respect to antiviral activity by heat (Fig. 1c) or trypsin treatment were insensitive to dsRNA. In addition, interferon treatment of Krebs cells of the line in use in this laboratory, which are very insensitive to interferon, did not enhance the sensitivity of EMC RNA translation in the cell-free system to dsRNA (data not shown).

The increased sensitivity to dsRNA reported here for cell-free systems from interferon-treated L cells raises the possibility that the enhanced inhibition of translation seen in cell-free systems from interferon-treated, vaccinia¹⁰, EMC¹¹ and mengo¹⁸ virus-infected L cells is triggered by the synthesis of small amounts of viral dsRNA. The events observed in the intact interferon-treated, vaccinia-infected L cell are consistent with this^{8,9}: both host and viral protein synthesis are inhibited (although whether by the same or different mechanisms is not yet clear), the cell dies and little virus is produced. A similar sequence of events occurs although more slowly in the interferon-treated EMC-infected L cell¹¹ (M. Esteban, D. R. Tovell, and I. M. K.,

unpublished). Double-stranded RNA has been detected in extracts from vaccinia-infected cells⁷ and may play a part in the replication of RNA viruses such as EMC. It is still not clear, however, that dsRNA as such exists in the intact cell¹⁹ and our initial attempts to detect a dsRNA fraction inhibitory to protein synthesis in cell-free systems from interferon-treated, vaccinia-infected L cells have been unsuccessful (L. A. B. and T. Hunt, unpublished). A direct connection between the effect of dsRNA and of infection on protein synthesis in the interferon-treated cell, therefore, remains to be established, but the copurification of the antiviral and dsRNA toxicity-enhancing properties of interferon preparations²⁰ strongly suggests that they are related.

Our results demonstrate an enhanced sensitivity of protein synthesis to dsRNA which could play a part in determining the fate of interferon-treated cells after infection with a variety of different viruses, possibly reflecting a mechanism basic to interferon action. If nothing more, they indicate a basis for the enhanced toxicity of dsRNA in the interferon-treated cell and provide further direct evidence for an interferon-mediated change in the protein synthetic apparatus of the cell.

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Excess males among siblings of Australian antigen carriers

REPEATED studies have shown that Australia antigen carriers tend to group in families and there is considerable evidence that susceptibility to chronic asymptomatic infection producing Australia antigen (Au, hepatitis B antigen) is inherited as an autosomal recessive trait¹⁻³. The occurrence of asymptomatic carriers of Au varies in different populations, ranging from 0.1% in the United States and northern Europe to 24% in the Melanesian population of the Lau people on Malaita, as measured by immunodiffusion⁴. The frequency of Au carriers is relatively high in Oceania and particularly high in Melanesian populations^{5,6}. If this trait is genetically determined, there would be a greater number of individuals who are either homozygous or heterozygous for the proposed recessive gene, *Au*¹, in populations where the trait is common. Blumberg has suggested that these individuals may possess a selective advantage in certain environments⁷.

In 1960, Dr William Davenport confirmed earlier observations concerning an excess of males on the island of Santa Cruz in the British Solomon Islands Protectorate. He suggested that this alteration in sex ratio might be the result of selection by an infectious disease (personal communication). Blumberg⁸ reported that there was a deficiency of male offspring of certain matings when one of the parents had Au, that is, that Au is related to alteration in sex ratio. Here we report finding an excess of males among siblings of Au carriers in a Melanesian population.

During July, August and September of 1973, the population of the 15 villages in Graciosa Bay, Santa Cruz Island, Eastern District, British Solomon Islands Protectorate (BSIP) was tested for Australia antigen by immunodiffusion. Eight hundred and fifty-two people, comprising more than 95% of the total resident population of all ages were bled from a finger or toe prick into capillary tubes. None of these people had overt hepatitis. The serum was separated from the red cells and tested in a seven hole pattern using rabbit anti-Australia antigen (A5 and 6) in the centre well and control serum, from an asymptomatic blood donor which contained the antigen, in the top and bottom wells. The plates consisted of 1.1% agarose in 8.2 veronal buffer and were read at 24, 48 and 72 h (ref. 9). A census of the people living in Graciosa Bay and their immediate families was compiled. This included the names and present location of the mother, father and live-born siblings of all residents.

The frequency of Au carriers was 4.8%. Forty-one individuals with Au were distributed in 35 groups of siblings. Three groups contained more than one carrier. The sex ratio of the siblings of Au positive individuals and matched negative controls are shown in Table 1. The controls were matched by sex and within one year of age and had no first degree relatives who were Au carriers. The first appropriate individual listed in the census after the Au carrier to be matched was accepted as the control. The analysis includes all live-born siblings regardless of subsequent death or absence from the village. The ratio of males to females among the Au carriers approaches 2:1. This excess of males among carriers has been observed previously¹⁰ and presumably is not dependent on any special conditions existing on Santa Cruz. The number of males per 100 females among the siblings of the control group is 98. The ratio of males to females among the siblings of Au carriers is 161:100. When these data are tested by Fisher's exact test (Table 2), the result indicates that the observed distribution is not likely to occur by chance. Thus, the observed sex ratio represents a significant excess of males among the siblings of Au carriers. This observation has important genetic, cultural and biological implications.

The report on the census of the population, 1970 by the Western Pacific High Commission, (BSIP) (ref. 11) contains

Table 1 Sex ratio of siblings of Au carriers and negative controls

	Total	Female	Male	No. of males per 100 females
Au carriers	41	14	27	193
Siblings of Au carriers	146	56	90	161
Siblings of Au negative controls	180	91	89	98

Table 2 Fisher's exact test (two-tailed)

	Female	Male	
Siblings of Au positives	56	90	$P = 0.0159$ this occurrence
			$P = 0.0364$ this occurrence or more extreme
Siblings of Au negatives	91	89	

considerable comment on the high incidence of males compared with females in the Solomon Islands, particularly in Melanesian populations. In 1970 there were 530 males per 1,000 Melanesians. There were 526 males per 1,000 in the 0-14 yr age group. The mechanism of this selection is obscure. It is reasonable to assume however, that males have had a reproductive advantage in Santa Cruz. Although there is an excess of males living on Santa Cruz, there are excess women available for marriage in the Reef Islands; these women are brought to Santa Cruz as brides. The keeping of concubines¹² has been practised until recently and there has been a high mortality among women of childbearing age in the Solomons¹¹, resulting in men frequently having more than one wife. In the Santa Cruz population, in matings which can produce Au carriers, there is apparently a sex related effect which reduces the number of live born female offspring. We suggest that because Australia antigen families have a higher proportion of males who have had a reproductive advantage and may be capable of transmitting the Au trait, the Au trait would tend to increase in the population resulting in the observed high frequency of both Au carriers and males in the Santa Cruz and possibly other Melanesian populations. The question of whether the mechanism of the transmission of the Au trait which results in family clustering is genetic or not is irrelevant to this model for Au carrier amplification.

Davenport has described trade relationships which have existed between the Outer Eastern Islands¹². The one-way travel of women from the Reef Islands to Santa Cruz in exchange for bride prices consisting of red feather money and food, was basic to the economy and trade relationships of the entire island group. The shortage of women on Santa Cruz, due in part to the excess masculinity in Au families, must have enhanced this trade. The removal of women to Santa Cruz also reduced the population expansion in the Reef Islands which have limited resources for the support of a growing population. Thus, the economy of an entire island group may to some degree be affected by selective pressures which are apparently related to the interaction between genetic factors and the epidemiology of an infectious agent.

The mechanism of prenatal selection among siblings of Au carriers is not clear. It may be directly related to foetal mortality resulting from infections with Au or the infectious agent associated with Au, or it may be due to maternal rejection of infected fetuses. It could also be the result of developmental or maternal-foetal interactions which are only indirectly associated with the production of Au carriers.

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Adenyl cyclase stimulation by aspirin in rat gastric mucosa

ASPIRIN consumption is associated with gastric mucosal erosions^{1,2} and upper gastrointestinal haemorrhage³⁻⁵, and has been postulated as a factor in the development of chronic peptic ulcer^{6,7}. It has been reported to cause chronic gastric ulcer in rats⁸. There is, however, no real understanding of the mechanism of these injuries. Menguy *et al.*⁹ suggested that the damage to the gastric mucosa results from lack of energy (ATP) for cell function. The reported antagonism of cholera enterotoxin by aspirin in the rat intestine¹⁰ might have been mediated through the inhibition of adenyl cyclase. We have now found, however, that aspirin can stimulate the activity of this enzyme in rat gastric mucosa.

Male Holtzman rats (200–300 g) were fasted overnight and then given aspirin by stomach tube (150–300 mg kg⁻¹ body weight, homogenised in 1% methyl cellulose). Control rats were given an equal volume of 1% methyl cellulose alone. After an appropriate time (5 min to 5 h) the rats were killed and mucosa was removed with a glass microscope slide; this and all subsequent manipulations were done at 2°–4° C. A 10% w/v homogenate of the mucosal scrapings was prepared in Tris–Mg²⁺ buffer, pH 7.5, in an all-glass homogeniser. This was centrifuged at 1,000g, and the precipitate was used for all assays.

Adenyl cyclase activity was determined from the rate of formation of 3',5'-cyclic AMP from ¹⁴C-ATP, (Amersham/Searle) specific activity 1.03 mCi mmol⁻¹, according to a

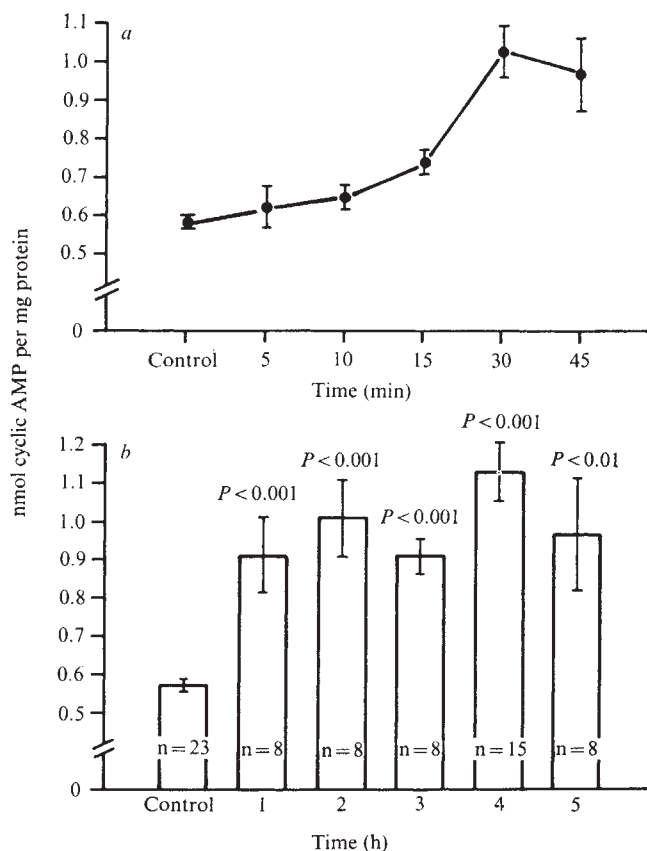


Fig. 1 *a*, Short term (5–45 min) effect of oral aspirin (300 mg kg⁻¹ body weight) on rat gastric mucosal adenyl cyclase. Each point represents a mean ± s.e.m. for three rats. Stimulation of adenyl cyclase becoming apparent within 5 min after oral administration. *b*, Long term (between 1–5 h) results. Adenyl cyclase activity is expressed as nmol cyclic AMP per mg protein, represented as bars. Each bar shows mean value of adenyl cyclase ± s.e.m. (n is the number of rats). All values are highly significantly different from the control. Various dose schedules (75–300 mg kg⁻¹ body weight) of aspirin were tried and a dose of 300 mg kg⁻¹ of body weight was selected as the optimal dose. 150 mg kg⁻¹ aspirin gave comparable results after 30 min of oral administration.

modification of the procedure of Krishna *et al.*¹¹. Enzyme activity was expressed as nmol of 3',5'-cyclic AMP produced per minute per mg protein as determined by the method of Lowry *et al.*¹².

Gastric mucosal homogenate (1,000g precipitate) as described above was prepared from 24 rats, in six groups of four. Aspirin in methyl cellulose was added to the gastric mucosa of each of four rats at concentrations of 0.3, 0.6, 0.9, 1.2 and 1.8 mg per 0.38 ml of reaction mixture. Four mucosae treated with equal volumes of methyl cellulose served as controls. The rest of the procedure for measuring adenyl cyclase activity was as described above.

The effect of aspirin (300 mg kg⁻¹) became apparent 5 min after the oral dose (Fig. 1). Stimulation of adenyl cyclase was maximum 4 h after the oral dose and was significantly different from the control value, as shown by Student's *t* test (*P* 0.001). Results were also significantly different from control after between 1 and 5 h, but not significantly different from each other.

Figure 2 shows that there is a direct correlation between aspirin concentration and the degree of adenyl cyclase activity. The activity seemed to increase linearly with aspirin concentration up to 1.8 mg of aspirin in 0.38 ml of reaction mixture.

The report that aspirin counteracted the effect of cholera toxin on the intestine of rat¹⁰ suggested a decrease in mucosal adenyl cyclase activity, and a similar decrease was expected

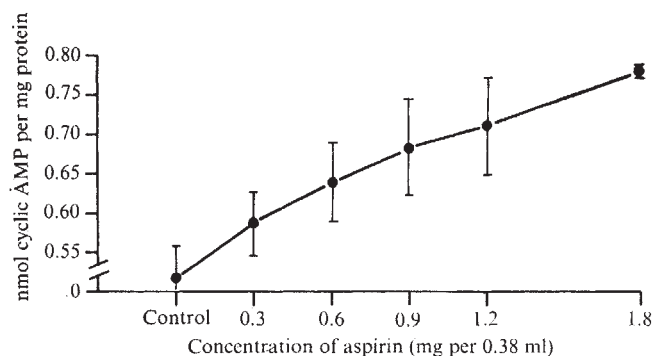


Fig. 2 Effect of aspirin *in vitro* at various concentrations (0.3–1.8 mg per 0.38 ml of reaction mixture) on rat gastric mucosal homogenate (1000g precipitate) adenylyl cyclase activity. Each point represents a mean $\pm$ s.e.m. of mucosae of four rats. A linear relationship between aspirin concentration and adenylyl cyclase activity is evident.

after administration of aspirin. Since cyclic AMP was shown to decrease gastric acid secretion^{13–15}, the lowering of adenylyl cyclase activity after aspirin might have explained the mechanism of injury by aspirin. But our experiments refute this contention. If one could correlate these findings and if one believes reports that cyclic AMP stimulates gastric acid secretion^{16–19}, the stimulation of adenylyl cyclase by aspirin may explain the mucosal injury. It is also possible that the stimulation exhausts the ATP stores⁹ quickly, thus causing mucosal injury.

Davenport^{1,2} demonstrated that aspirin damages the gastric mucosal barrier and causes hydrogen ion back diffusion, as judged by the development of hypoacidity in a gastric pouch after administration of aspirin. If one postulates^{13–15} that cyclic AMP inhibits gastric acid secretion, this may be another explanation for the hypoacidity seen after injury by aspirin.

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Cyclic AMP, ATP and cell contact

DENSITY-DEPENDENT¹ or contact-inhibited² cells stop dividing when they reach confluency; however, transformed cells do not exhibit this type of growth control³. Several experiments suggest that adenosine 3',5'-cyclic monophosphate (cyclic AMP) is involved in regulating the cell division of cultured fibroblasts. The dibutyl derivative of cyclic AMP inhibits proliferation of transformed cells⁴, and the steady state levels of cyclic AMP in normal density-dependent fibroblasts are higher than the corresponding virus and spontaneous transformed cell lines^{5,6}. There are, however, discrepancies between experiments which have attempted to compare the cyclic AMP levels of logarithmically growing with quiescent, density-inhibited 3T3 cells. Experiments in our laboratory showed that cyclic AMP levels of 3T3 cells do not change over the cell density of 1×10^4 – 6×10^4 cells per cm^2 (ref. 5). These results have been confirmed by others using the same cell system⁷. Otten *et al.*^{6,8}, however, reported that cyclic AMP levels of logarithmically growing 3T3 cells are lower than those of confluent, quiescent cells; Heidrick and Ryan⁹ reported similar results using L cells.

In an attempt to reconcile those contradictory data, we have thoroughly investigated the relationship between cell density and the cyclic AMP levels of mouse 3T3 cells. We have also studied deoxyglucose transport and cellular ATP levels as possible density-dependent phenomena. Measurements were made at sparse cell densities (no cell-cell contact), after cell contacts were established and again after growth to a confluent monolayer. A critical membrane-mediated event seems to occur at the cell-cell contact state (before confluency), resulting in decreased glucose transport, decreased ATP and increased cyclic AMP. Such changes may prime the normal cell for eventual density-dependent growth inhibition. Transformed cells which do not exhibit regulated inhibition of growth show little change in these biochemical parameters when compared at the three cellular densities.

Swiss 3T3, 3T6 and PY3T3 cells (a gift of Dr Howard Green) and BALB 3T3 cells and SV3T3 cells (a gift of Dr G. J. Todaro) were routinely grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum (Colorado Serum Co.) and penicillin-streptomycin (100 U ml^{-1} and 100 $\mu\text{g ml}^{-1}$, respectively). Cyclic AMP was measured by Gilman's method¹⁰. Cells were prepared for assay by washing twice with phosphate-buffered saline (warmed to 37° C), then adding cold (4° C) 5% trichloroacetic acid (TCA). The TCA supernatant, obtained by centrifugation, was extracted five times with 5 volumes of water-saturated ether. Samples were then applied on small Dowex-1-formate columns (0.4 $\times$ 3 cm) equilibrated with water. Columns were washed with 10 ml of water, and then cyclic AMP was eluted with 1 N formic acid. Eluates were lyophilized to dryness and redissolved in 0.2 or 0.4 ml of water. A sample of 0.05 ml was used for each cyclic AMP assay. Recovery of cyclic AMP, which was measured using ³H-cyclic AMP, was more than 90%, irrespective of total amounts of cyclic AMP (from 0.5 to 20 pmol). Another purification method using Dowex 50-H+ (ref. 8) was tested, but we found that the resin releases a substance which strongly inhibits cyclic AMP binding and so discarded this method. Internal standards and susceptibility to degradation by cyclic nucleotide phosphodiesterase were used routinely to verify that we were measuring cyclic AMP.

³H-deoxyglucose uptake was measured by the procedure of Martin *et al.*¹¹ after previous incubation at 37° C for 45 min in glucose-free Hanks salt solution. Cellular ATP was measured using a firefly lantern extract¹². Cells were washed three times with warm phosphate-buffered saline, and then cold 50% ethanol was added. The cells were scraped from the dish with a rubber policeman and mixed by a vortex. After at least 30 min in an ice bath, the suspension was directly used for determination of ATP. Protein was assayed according to Lowry *et al.*¹³.

Normal Swiss 3T3 cells grow in culture to 4×10^4 – 8×10^4 cells cm^{-2} at which density the cellular monolayer becomes quiescent as measured by DNA synthesis and cell division. The logarithmic phase of the growth cycle (under our laboratory conditions) extends to a density of 2×10^4 cells cm^{-2} ; at this density proliferation begins to plateau and eventually stops at 4×10^4 – 8×10^4 cells cm^{-2} . The relation between cell density and the intracellular cyclic AMP level is illustrated in Fig. 1. Since at least 10^6 cells were needed for each cyclic AMP assay, roller bottles were used for the lighter cultures and two to six 60- cm^2 plastic tissue culture dishes were used for the heavier cultures. Cells were plated at various concentrations (all other conditions being constant) and grown for 24 h. The cyclic AMP level and cell number were then determined. The basal level of cyclic AMP was low at sparse 3T3 cell density and began to increase at about 3×10^3 cells cm^{-2} (Fig. 1). Cyclic AMP then quickly increased to a stationary concentration that was twice the basal level of the sparse, noncontacted normal cells. This increase occurred during log phase growth at a density (3×10^3 cells cm^{-2}) that preceded confluency and the total cessation of cell division.

ATP levels of 3T3 cells at various densities were also investigated, and contrary to the results observed with the cyclic AMP levels, the amount of ATP per 10^6 cells decreased, reaching a minimum (about half of the maximum) after cell contact was established.

To preclude the possibility that cell volume changed as density increased, we measured the cell protein of 3T3 and transformed cell lines as they grew to confluency. Table 1 is a summary of that data and shows little effect of cell density on protein content of the cell lines studied. There were, however, large differences between mg protein per 10^6 cells of the various normal and transformed cell lines. Calculation of ATP concentrations using protein or cell number indicated differences in the ATP levels of normal and transformed cells.

PY3T3 and 3T6 cells had ATP levels similar to growing (precontact) Swiss 3T3 cells, but once the normal 3T3 cells touched and the ATP level decreased, there was a significant difference between the transformed and normal 3T3 cells. A similar trend was observed with the BALB mouse-derived normal and transformed cell lines. A previous report that growing 3T3 cells had slightly more ATP than quiescent 3T3 cells¹⁴ supports our present data, because the earlier comparison was made with cells that had established contact, and maximal differences between growing and confluent 3T3 cells were observed only with precontacted 3T3 cells.

Deoxyglucose transport followed a pattern similar to the ATP level in that uptake decreased after cell contact. Experiments using chick fibroblasts¹⁵, BALB 3T3 cells¹⁶, and mouse embryo cells¹⁷ have shown that deoxyglucose transport is inhibited at relatively low cell densities. Our experiments, using Swiss 3T3 cells, show that deoxyglucose transport is inhibited at a density as low as 2×10^3 cells cm^{-2} . Initial cell-cell contacts are established at 10^3 cells cm^{-2} . These data suggest that inhibited cellular transport is one of the first biochemical parameters affected by cell contact. Thus, the increase in cellular cyclic AMP may be a critical (although not necessarily primary) event and might, as previously suggested¹⁸, serve as a cellular signal which responds to the availability of crucial nutrients.

Increasing cyclic AMP levels could result from either a stimulated adenylate cyclase or an inhibited phosphodiesterase activity. The activity of adenylate cyclase, a plasma membrane enzyme, increases (both basal and adrenaline stimulated) as a function of 3T3 cell density¹⁹. But, Russell and Pastan²⁰ suggested that a membrane-associated cyclic AMP phosphodiesterase exerts a substantial effect on the cellular cyclic AMP level. The biological mechanism of the ATP change is unknown and any possible relationship between levels of ATP and cyclic AMP remains to be established.

These experiments settle the controversy concerning the role of cyclic AMP in the contact inhibition or density-dependency phenomenon. Otten *et al.*^{6,8} suggested that cyclic AMP mediates contact inhibition and that an increase of the basal level of cyclic AMP of the 3T3 cell occurred at confluency, but earlier we could detect no change in the basal level of cyclic AMP as 3T3 cells grew to confluence⁵. This controversy is now resolved by our finding that 3T3 cyclic AMP increases in response to cell contact which occurs at a density of 3×10^3 cells cm^{-2} . Little change in cyclic AMP level occurred at confluency. We missed the increase in cellular cyclic AMP before⁵ because the plating density of the 3T3 cells exceeded the cell density at which the cellular cyclic AMP increases from its initial level. Thus, we observed only a constant and increased cellular cyclic AMP level over the density of 10^4 cells per cm^2 to 6×10^4 cells per cm^2 . This change in the cyclic AMP level is critical in contact inhibition, as Otten *et al.*^{6,8} and Heidrick and Ryan⁹ concluded, although it does not, as they suggested, directly participate in or coincide with cessation of 3T3 cell division as cells become confluent. We suggest that this biochemical change is a prerequisite for contact inhibition of growth by showing that cyclic AMP

Table 1 ATP levels of several normal and transformed lines

Cell line	Protein (mg per 10 ⁶ cells)	Cell density (cells cm ⁻²)	ATP (nmol per 10 ⁶ cells)	ATP (nmol per mg protein)
Swiss-derived:				
3T3	0.48	1.5 × 10 ³ (precontact)	18.0 ± 1.8	37.5
	0.48	1.5 × 10 ⁴ (contact)	12.6 ± 1.7	26.3
	0.44	4.1 × 10 ⁴ (confluent)	10.9 ± 0.8	24.7
PY3T3	0.39	2.5 × 10 ³ (precontact)	14.5 ± 1.3	37.2
	0.43	2.0 × 10 ⁴ (contact)	14.4 ± 0.9	33.5
	0.41	5.0 × 10 ⁴ (confluent)	15.8 ± 0.8	38.5
3T6	0.37 ± 0.04	3.3 × 10 ³ (precontact)	11.6 ± 1.2	31.4
		4.4 × 10 ⁴ (contact)	12.0 ± 0.5	32.4
		1.1 × 10 ⁴ (confluent)	11.5 ± 0.5	31.1
BALB-derived:				
3T3	0.44 ± 0.04	1.4 × 10 ³ (precontact)	11.5 ± 0.5	26.1
		4.4 × 10 ⁴ (contact)	9.9 ± 0.2	22.5
		8.3 × 10 ⁴ (confluent)	8.7 ± 0.3	19.8
SV3T3	0.16 ± 0.02	3.0 × 10 ³ (precontact)	4.7 ± 0.2	29.4
		5.5 × 10 ⁴ (contact)	4.2 ± 0.3	26.3
		1.4 × 10 ⁵ (confluent)	4.3 ± 0.3	26.9

Protein determinations were performed on monolayer cultures at various densities after washing three times with phosphate-buffered saline. The cells were then solubilised in 1 N NaOH and protein was measured according to Lowry *et al.*¹⁴. ATP levels were measured by the method of Strehler and McElroy¹². Single points are the average of triplicate determinations. Variation is expressed as $\pm$ s. d. when at least six separate determinations were done.

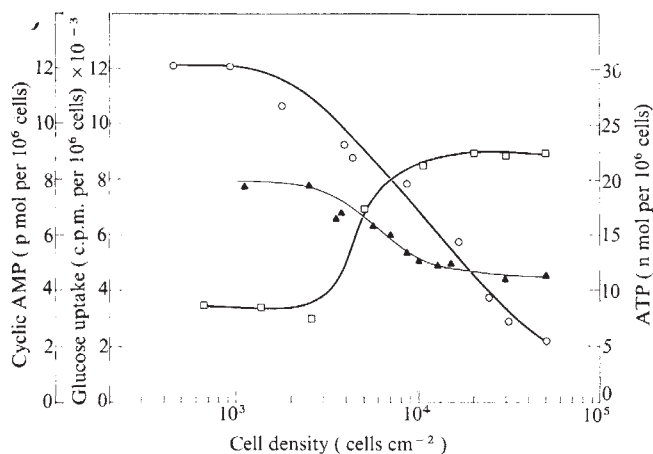


Fig. 1 Cyclic AMP levels ($\square$), ATP levels ($\blacktriangle$), and deoxyglucose transport ($\circ$) were measured as described in the text. These data points are the averages of triplicate determinations derived from one experiment.

increases at cell contact before the accumulation of 3T3 cells in a quiescent monolayer mechanisms.

Siefert and Paul²¹ found that cyclic AMP levels did not vary as a function of cell density; increased cyclic AMP was observed after 3T3 cells stopped dividing, regardless of density. They attributed the increase to the depletion of a serum factor necessary for continued proliferation. Our measurements were made 24 h after cell plating and were presumably not affected by serum or medium depletion. It is conceivable that both cell contact and serum factors could affect cyclic AMP levels, possibly through cell transport mechanisms.

These and other studies²²⁻²⁵ suggest that an important membrane-associated event in the contact inhibition or density-dependent inhibition of growth precedes the accumulation of normal cells in a confluent monolayer. Most likely, all membrane parameters that change as a function of normal cell contact—nutrient transport¹⁵⁻¹⁷, distribution of intramembranous particles²², membrane fluidity²³, plasma membrane enzyme activity²⁴ and cellular agglutinability²⁵—as well as the intracellular concentrations of cyclic AMP and ATP, signify important cellular changes culminating in inhibited cellular proliferation. But, each of these changes taken alone is probably not sufficient to induce true density-dependent inhibition of growth. Future studies should describe the membrane-associated signal which initiates these biochemical events and clarify relationships among the cellular processes that result in regulated cell division.

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α - and β -Retinyl acetate reverse metaplasias of vitamin A deficiency in hamster trachea in organ culture

DEFICIENCY of vitamin A causes a well-defined lesion, namely keratinised squamous metaplasia, in tracheobronchial epithelium¹. In this lesion, the normal columnar ciliated and mucus cells of the epithelium, which depend on vitamin A for their formation, are totally replaced by squamous cells which produce keratin. The mechanism of action of vitamin A in controlling this normal differentiation of ciliated and mucus cells is still unknown. We report here an *in vitro* system for studying this process, using organ culture of hamster tracheas in a chemically defined, serum-free medium. Growth of tracheas in this medium without vitamin A causes keratinised squamous lesions. Addition of vitamin A to the organ cultures after development of such lesions causes reversal of the process of keratinisation and replacement of the squamous cells by columnar ciliated and mucus cells.

It has previously been noted² that cultivation of mouse prostate in chemically defined medium without vitamin A resulted in squamous metaplasia of the prostatic secretory epithelium and that inclusion of vitamin A in the defined medium completely prevented this metaplasia. The addition of the vitamin A antagonist, citral, to tracheal organ cultures has also been reported to cause squamous epithelial lesions, but simultaneous inclusion of vitamin A in the medium prevented their formation³. In the present study, we have used vitamin A acetate (β -retinyl acetate) to reverse lesions of vitamin A deficiency, rather than prevent their formation. Moreover, we have found that the α -retinyl isomer of vitamin A (Fig. 1), previously believed to be an essentially inactive form^{4,5}, is also active (as α -retinyl acetate) in our *in vitro* test system.

Syrian golden hamsters were maintained from birth on a diet deficient in vitamin A (ref. 6) (General Biochemicals, Chagrin Falls, Ohio). The animals were weaned at 21 d and killed at 30–33 d. At this time the hamsters were still gaining weight and did not yet show signs of severe vitamin A deficiency; the tracheal epithelium was generally low columnar

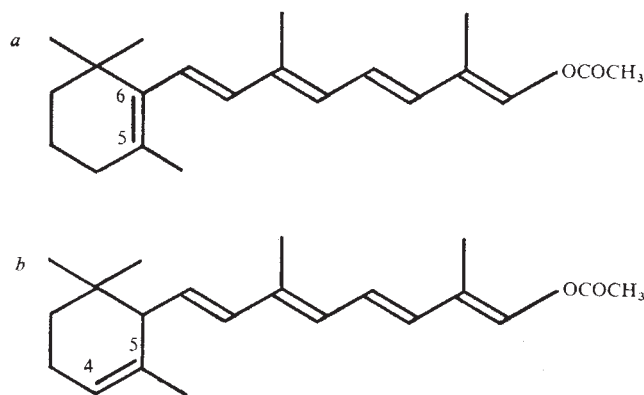


Fig. 1 Structures of (a) β and (b) α -retinyl acetate. The double bond in the 5-6 position of the cyclohexene ring of the naturally occurring β -isomer has been shifted to the 4-5 position in the α -isomer.

or cuboidal, with patches of squamous metaplasia only rarely found. The animals were randomly grouped for treatment, and their tracheas were removed by sterile technique. Each trachea was opened from the larynx to the carina along the membranous dorsal wall and placed into a 60 $\times$ 15 mm culture dish containing 2 ml of medium (CMRL-1066), (ref. 7); with crystalline bovine insulin, 1 μ g ml⁻¹; hydrocortisone hemisuccinate, 0.1 μ g ml⁻¹; glutamine, 2 mM; penicillin, 100 units ml⁻¹; and streptomycin, 100 μ g ml⁻¹, added). All tracheas were cultured at 37° C for 4 d in the above medium, which does not contain vitamin A, serum, or any added protein other than insulin. All cultures were gassed with 50% oxygen, 45% nitrogen, and 5% carbon dioxide in a sealed box, which was gently rocked approximately 12 times min⁻¹ to allow the trachea contact with both gas and medium. After 4 d, some tracheas were collected and the rest were cultured for an additional 6 d (before collection) in the above medium, also containing either a, no added vitamin A; b, β -all-*trans*-retinyl acetate (0.25–1.0 μ g ml⁻¹); or c, α -all-*trans*-retinyl acetate (0.25–1.0 μ g ml⁻¹). Properties of α -retinyl acetate were as follows: melting point=41–42.5; by ultraviolet analysis $\lambda_{\text{max}}^{\text{EtOH}}=311$ nm, $\epsilon=64,900$. No β -isomer was detectable in the α -retinyl acetate by nuclear magnetic resonance (NMR) analysis, which is capable of detecting as little as 0.2% β -retinyl acetate impurity; details of these analyses will be published elsewhere. β and α -retinyl acetate were dissolved in dimethyl sulphoxide and added freshly to the cultures at the time of media change, which was done 4 times a week. The final concentration of dimethyl sulphoxide never exceeded 0.1%; an equivalent amount was added to all control cultures. At the time of collection, all tracheas were fixed in 10% buffered formalin and embedded in paraffin. Cross sections of 5 μ m were made through the mid-portion, stained with haematoxylin and eosin, and evaluated independently by two observers. The status of the epithelium was graded with respect to both the extent of squamous metaplasia and the presence or absence of keratin.

The results of over 200 tracheal cultures are shown in Table 1 and demonstrate the progression of keratinising squamous metaplasia in culture, as well as its reversal on addition of β - or α -retinyl acetate to the medium. After 4 d *in vitro* in medium without vitamin A, 56% of cultures exhibited some squamous metaplasia and 52% had produced keratin. After 10 d *in vitro* without vitamin A, further progression resulted in 84% of the cultures showing squamous metaplasia and 76% showing keratinisation. Addition of β or α -retinyl acetate to the cultures from days 4–10 effected a reversal of squamous metaplasia, so that less than 25% showed squamous metaplasia at 10 d. In contrast to the marked or severe squamous metaplasia seen in 32% of the cultures on day 4 (before treatment with vitamin A), only 3% of the cultures had this extent of metaplasia after 6 d of treatment with β -retinyl acetate. Further, the keratinisation present in 52% of cultures at day 4 was reversed by β -retinyl acetate treatment in every case; keratinisation was reversed in all but two cultures treated with α -retinyl acetate.

This organ culture method will facilitate the study of vitamin A analogues for their capability to effect normal differentiation of tracheobronchial epithelium. Since the assay measures the reversal of lesions caused by the absence of vitamin A, as well as inhibition of their formation, it seems that the effect of an analogue in this assay could not be attributable solely to a sparing or stabilising effect that it might have on endogenous vitamin A metabolism. Such a sparing effect on endogenous hormone metabolism has been suggested to explain the wide range of chemical structures which show insect juvenile hormone activity^{8,9}.

This assay clearly demonstrates that the α -isomer of vitamin A, previously reported to be almost inactive (as the aldehyde or alcohol) in supporting growth in the rat, has intrinsic ability to maintain normal differentiation in hamster tracheobronchial epithelium. This finding has been extended (unpublished results) in *in vivo* growth studies in our laboratory, which have demonstrated the capacity of α -retinyl acetate to sustain growth and life if given intraperitoneally to hamsters that would otherwise lose weight and die on a vitamin A-deficient diet. The potency of the α -isomer, however, is markedly less than that of the β -isomer in the hamster growth assay. It has also been shown that both α -retinoic acid and α -retinyl acetate have definite ability to promote growth in rats maintained on vitamin A-deficient diets¹⁰.

Our organ culture system may also prove useful to determine whether vitamin A and its analogues are capable of reversing squamous metaplastic or preneoplastic lesions of tracheobronchial epithelium that are induced by chemical carcinogens. Addition of excess vitamin A to prostatic organ cultures previously treated with 3-methylcholanthrene can suppress the hyperplastic and metaplastic response caused by this carcinogen in prostatic epithelium¹¹. It has also been shown that simultaneous administration of vitamin A to hamster tracheal organ cultures treated with benzo[*a*]pyrene can inhibit the hyperplastic response caused by the carcinogen¹²; however, in this case the inhibitory effects of vitamin A may be caused by inhibition of activation of the carcinogen¹³. In view of the hypothesis that human tracheobronchial epithe-

Table 1 Response of tracheal organ cultures to β -retinyl acetate or α -retinyl acetate

Treatment		Cultures with respective amounts of squamous metaplasia (%)				Cultures with keratin (%)	Total cultures
Day 1–4	Day 4–10	None	Mild	Marked	Severe		
No vitamin A	Collected day 4	44	24	17	15	52	46
No vitamin A	No vitamin A	16	32	28	24	76	62
No vitamin A	β -retinyl acetate	83	14	3	0	0	63
No vitamin A	α -retinyl acetate	76	14	8	2	3	59

Cultures were graded as to the percentage of their total epithelium showing squamous metaplasia on 4–8 cross sections from the middle of each trachea; if between 1% and 10% of the total epithelial length was squamous, it was graded as having mild squamous metaplasia; between 10–40% was graded as marked; and greater than 40% was graded as severe.

lium undergoes a prolonged series of metaplastic and pre-neoplastic changes before development of bronchogenic squamous cell carcinoma¹⁴, it would be of definite interest to know whether vitamin A and its analogues are capable of arresting or reversing the development of these lesions in organ culture.

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Dynamics and function of vitamin A compounds in rat retina after a small bleach of rhodopsin

THE vertebrate visual pigment, rhodopsin, is located in the outer segment disks of the rod photoreceptor cells, and consists of a chromophore, 11-*cis* retinal, bound to a protein, opsin. During light adaptation, 11-*cis* retinal is isomerised to the all-*trans* configuration. All-*trans* retinal is then reduced to retinol by an oxido-reductase in the rod outer segments¹⁻⁴, and retinol diffuses to areas adjacent to the rod outer segments, where it is esterified to form retinyl esters⁵⁻¹². During dark adaptation rhodopsin is regenerated as essentially the reverse processes occur; however, it is not known in what form (retinyl ester, retinol, retinal) the chromophoric group is re-isomerised to the 11-*cis* configuration.

This understanding of the vertebrate visual cycle is based upon experiments in which high light intensities were used, and most or all of the rhodopsin was bleached. It is not known whether release, reduction and esterification of the chromophore occur after a smaller fraction of rhodopsin is bleached. Here, we report that these reactions also occur when 10% of the rhodopsin in a dark-adapted rat eye is bleached by a single light flash, and that some of the chromophore may be

re-isomerised to the 11-*cis* configuration without reduction to retinol.

Our technique for determining the *in vivo* proportions of vitamin A compounds consists of injecting radioactive vitamin A compounds into vitamin A-deficient rats, and then cochromatographing extracts of eye tissues with known marker compounds on thin layer chromatography plates¹²; the amount of radioactivity which cochromatographed with each marker compound was taken as an indirect measure of the amount of that compound in the extract. The method was used to study the distributions and proportions of vitamin A compounds in the eye during and after adaptation to high light intensities¹². In this study we determined the proportions of vitamin A compounds in rat eyes, one of which was kept in darkness, while the other was exposed to a light flash which bleached approximately 10% of the rhodopsin present.

One eye of a dark-adapted albino rat was exposed to a 20 ms flash of monochromatic light (504 nm) which had an intensity of 4.2 mW cm⁻². The flash apparatus consisted of a light source which focused light through an interference filter onto an optic fibre 7 mm in diameter; the other end of the optic fibre was mounted in front of a piece of doubly ground glass and an electronically-actuated mechanical shutter. At successive intervals after the light flash, both eyes were removed and the extracts of them were cochromatographed with marker vitamin A compounds (11-*cis* and all-*trans* retinal, retinol, retinyl ester) on silica gel thin-layer plates with a solvent of 8 parts ethyl acetate:92 parts hexane. The percentage of radioactivity in a vitamin A compound was obtained by dividing the amount of radioactivity which cochromatographed with that compound by the total amount of radioactivity which cochromatographed with all of the marker compounds¹². Subtraction of the percentage of radioactivity in a compound in the dark-adapted eye from the percentage of radioactivity in that same compound in the light-exposed eye yielded the results shown in Fig. 1.

By 5 to 10 min after the light flash, 11-*cis* retinal had decreased to its minimum, and *trans* retinal and retinol had increased. The 8% difference between the experimental and control eyes in the concentration of 11-*cis* retinal reflects a bleach of approximately 10% of the rhodopsin because 11-*cis* retinal, present only as the chromophore of rhodopsin, comprises 80% of the vitamin A compounds in the dark-adapted rat eye¹². Using the measurements of Cone¹³ for the attenuation

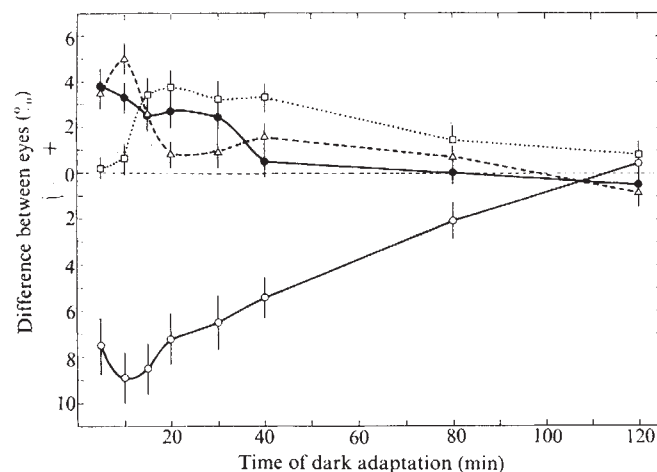


Fig. 1 Vitamin A compounds formed in the dark-adapted rat eye after a 20 ms flash of monochromatic light (504 nm). The ordinate scale is the difference between the percentage of radioactivity in a vitamin A compound in the flashed (experimental) eye and the percentage of radioactivity in that same compound in the dark-kept (control) eye. The abscissa scale is time after the light flash. Lines connect the mean values of four to six experiments; vertical lines indicate standard errors. □, Retinyl ester; △, retinol; ●, *trans* retinal; ○, 11-*cis* retinal.

and scattering of light by the rat cornea and lens and his measurements of the number of rods in the retina, we calculate that the rhodopsin of the entire retina should absorb 4.3×10^{13} light quanta. We can extract approximately 1 nmol of rhodopsin (6.0×10^{14} molecules) from the dark-adapted rat eye, and, assuming a quantum efficiency of bleaching of 0.65 (ref. 14), we should then expect to bleach about 5% of the rhodopsin by the light flash; considering the many sources of error in these measurements, this is in close agreement with our value of 10% inferred from the indirect detection technique.

By 10 min after the flash, *trans* retinal had started to decrease, retinol was at its peak concentration, and retinyl ester was increasing. As in adaptation to high light intensities, these results most probably reflect release of *trans* retinal from photolysed rhodopsin, reduction of retinal to retinol and esterification of retinol to fatty acids^{3,6-12}.

During 15 to 40 min after the flash there was regeneration of rhodopsin, as is evident from the increase in 11-*cis* retinal. During the same time, *trans* retinal decreased in concentration, but the concentration of retinyl ester remained stable. This result strongly suggests that some *trans* retinal was re-isomerised to the 11-*cis* configuration without being reduced to retinol. From 40 to 120 min after the flash, retinyl ester gradually decreased in concentration, while 11-*cis* retinal continued to increase; by 2 h after the flash, there was no longer any significant difference between the eyes in the proportions of vitamin A compounds. As in dark adaptation following extensive bleaching of rhodopsin, these results probably reflect hydrolysis of retinyl ester, diffusion of retinol into the rod outer segments, oxidation to retinal and isomerisation (at some point) to the 11-*cis* isomer^{3,7-12}.

In spite of the experimental variability, then, two important conclusions are suggested by these results: first, that release, reduction and esterification of the chromophore occur when 10% of the rhodopsin is bleached; and second, that isomerisation of the chromophore from the all-*trans* to the 11-*cis* isomer probably occurs as retinal. The latter conclusion is also supported by recent experiments with cattle retinae¹⁵ and rat retinae¹⁶.

As discussed elsewhere by one of us¹², the dynamics of vitamin A compounds in the eye (release, reduction and esterification of the chromophore and the reverse) may have one or some combination of three functions: (1) re-isomerisation of the chromophore; (2) minimising accumulation of reactive aldehyde groups and diffusional loss of retinol during light adaptation; and (3) confinement to and recycling of chromophore in the eye.

The present results argue against the first possibility—that re-isomerisation of the chromophore must occur in the retinol or retinyl ester form—since a 'short cycle' (release of all-*trans* retinal, re-isomerisation to the 11-*cis* form, and resynthesis of rhodopsin) seems to occur. To test the second possibility—that the 'long cycle' (reduction, esterification, hydrolysis, oxidation) is an overflow device which minimises accumulation of retinal and diffusional loss of retinol—would require determining whether a 10% bleach of rhodopsin is within the normal operating range of rod cells, and, if not, whether the long cycle occurs after even smaller bleaches of rhodopsin; the indications that some all-*trans* retinal is re-isomerised to the 11-*cis* form without being reduced to retinol might suggest that at still lower bleaching levels of rhodopsin, no reduction to retinol occurs at all. The third possible function—that of confinement and recycling of chromophore in the eye—seems especially important, not only during light adaptation, but also during the rod renewal process. In this renewal process, the rod disks (including rhodopsin) are synthesised *de novo* at the base of the outer segments, displaced apically, and finally digested by the pigment epithelium¹⁷⁻²⁰. Since the chromophore required for *de novo* synthesis of rhodopsin is not stored in the retina and must reach it through the pigment epithelium, the fraction of total vitamin A compounds in the eye which must be returned daily to the retina is $1/n$, where n is the number of

days required for complete rod renewal. Considering that rod renewal in the rat and monkey require, respectively, 9 and 12 d (refs 21, 22), the importance of a vitamin A transport mechanism is clear.

The chemical properties of vitamin A compounds and the locations of the enzymes mediating their interconversion seem especially well suited to such a function of confining the chromophore to the eye and transporting it within the eye. Thus, in addition to a reactive form, retinal, which forms visual pigment in the outer segments, there is a transport form, retinol, which is diffusible, penetrates cell membranes, and is formed in the outer segments; and there is a storage form, retinyl ester, which is nonreactive, nondiffusible and formed in areas adjacent to the outer segments.

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Difference in the cellular cholesterol to phospholipid ratio in normal lymphocytes and lymphocytic leukaemic cells

FLUIDITY of the fatty acyl chains of phospholipids in cell membranes is required for essential cellular activities, and the degree of fluidity can be influenced by cholesterol. Changes in the ratio of cholesterol to phospholipids can, therefore, affect the internal viscosity and molecular motion of lipids within membranes and this may function as a regulator in cell behaviour¹⁻⁴. Our studies of the surface membrane of normal lymphocytes and

Table 1 Cholesterol and phospholipid content of normal lymphocytes and lymphocytic leukaemia cells

Cell type	Total lipid (g $\times 10^{-12}$ per cell)	Cholesterol (mol $\times 10^{-15}$ per cell)	Cholesterol (g $\times 10^{-6}$ per mg lipid)	Phospholipids (mol $\times 10^{-15}$ per cell)	Phospholipids (g $\times 10^{-6}$ per mg lipid)*	Cholesterol: lipid - P molar ratio
Rat lymphocytes	4.4 $\pm$ 0.5	0.6 $\pm$ 0.05	53.5 $\pm$ 0.8	2.35 $\pm$ 0.3	442 $\pm$ 23	0.25 $\pm$ 0.01
Mouse lymphocytes	3.9 $\pm$ 0.5	0.55 $\pm$ 0.1	54.7 $\pm$ 2.7	2.1 $\pm$ 0.3	403 $\pm$ 33	0.26 $\pm$ 0.03
YAC leukaemia	16.8 $\pm$ 0.8	2.2 $\pm$ 0.2	51.8 $\pm$ 3.3	11.7 $\pm$ 0.9	550 $\pm$ 40	0.19 $\pm$ 0.01
EL4 leukaemia	12.7 $\pm$ 0.9	1.7 $\pm$ 0.1	51.6 $\pm$ 3.4	9.9 $\pm$ 1.0	604 $\pm$ 77	0.17 $\pm$ 0.01

Standard deviations are given for the mean value of five or six determinations for each type of cell. Normal lymphocytes from the lymph nodes of 6–8-week-old animals were collected by teasing the tissue apart in a cold solution of tricine-buffered saline (pH 7.4) and allowing the pieces to sediment. The cells were then washed three times with cold tricine-buffered saline. Both ascites tumours were generally inoculated intraperitoneally at 10^6 cells per adult mouse, the cells collected 12–14 d later and washed three times in cold tricine-buffered saline. Lipids were extracted by chloroform-methanol (2:1, v/v), as described⁹. Stirring was continued overnight at 4° C and there was no significant increase in yield of lipid phosphorous by a second extraction of the residue obtained after filtration. The chloroform-methanol extracts were washed as described¹⁰ with 0.1 M KCl in the upper phase. Lipid phosphorous was determined by the method of Ames¹¹ after digestion of the sample with an ethanolic solution of Mg (NO₃)₂. Free cholesterol was separated from cholesterol ester by column chromatography on silicic acid, and the two components were then assayed separately by the FeCl₃ method¹². The cholesterol ester was only 0.5–1 % of the total lipid.

* Assuming an average phospholipid molecular weight of 775.

lymphocytic leukaemic cells have shown that the surface membrane of the leukaemic cells has a lower rotational mobility of concanavalin A binding sites^{5,6}, and a higher fluidity of the lipid layer^{6,7}. We have now found that these differences in fluidity are associated with a difference in the cholesterol to phospholipid ratio in these cells.

Normal lymphocytes obtained from the lymph nodes of A and C57BL mice and CR/RAR rats all gave similar results. These cells contained 70–85% thymus-derived cells. Normal B lymphocytes were obtained from the lymph nodes of C57BL nude mice. The lymphocytic leukaemic cells used were from two thymus-derived mouse ascites tumours, one from A strain mice (YAC) used before^{5–7} and the second (EL4) from C57BL mice⁸.

The cellular content of total lipids, free cholesterol and phospholipids was determined in the different cells (Table 1). The leukaemic cells were larger than the normal lymphocytes, and contained a larger amount of total lipids, cholesterol and phospholipids per cell. In both types of cell, however, cholesterol was 5–6% of the total lipid, so that the normal lymphocytes and leukaemic cells had about the same amount of cholesterol per mg dry lipid. Phospholipids were 40–45% and 55–65% of the total lipids in normal and leukaemic cells, respectively, so that the normal lymphocytes contained less phospholipid. The proportion of phospholipids in the B lymphocytes from nude mice was about the same as that in the normal lymphocytes which contained 70–85% thymus-derived cells. The molar ratio of cholesterol to phospholipids (Table 1) was therefore significantly smaller ($P < 0.001$) in the thymus-derived leukaemic cells than in the thymus-derived or B normal lymphocytes.

Studies of leukaemic cells collected at different times after inoculation of 2.5×10^6 cells per animal have shown that cells with a decreased rate of cell division, 12–14 d after inoculation, had a similar cholesterol to phospholipid ratio to that of cells in the logarithmic phase of growth. The decreased cholesterol to phospholipid ratio that we have found in the mouse leukaemic cells that were larger than the normal lymphocytes, has also been observed with human lymphocytic leukaemias where the cells were smaller than the normal lymphocytes⁹. It is therefore unlikely that the difference in the ratio between normal and leukaemic cells is only due to a difference in cell size. Although there is a decrease in the cholesterol to phospholipid ratio, it is interesting that cholesterol is synthesised more rapidly in lymphocytic leukaemic cells than in normal lymphocytes¹³, presumably due to a loss of the feedback control that has been found with hepatomas¹⁴.

Our results indicate that the higher fluidity of the lipids in the surface membrane after malignant transformation of normal lymphocytes^{6,7} is associated with a decrease in the molar ratio of cholesterol to phospholipids, and not with a significant decrease in the amount of cholesterol per mg lipid. The larger cholesterol to phospholipid ratio in the normal lymphocytes can

explain the lower fluidity of their surface membrane lipids. We suggest that differences in this ratio may be involved in the regulation of growth and differentiation in normal and malignant cells.

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Quantum conductance changes in lipid bilayer membranes associated with incorporation of acetylcholine receptors

CONSIDERABLE attention has focused on the isolation and characterisation of the nicotinic acetylcholine (ACh) receptor from electroplax^{1–4}. This receptor is identified by its ability to bind cholinergic ligands, and its molecular weight and amino acid content have been estimated^{5,6}. Similar extraction procedures have also been applied to nicotinic receptors from mammalian brain⁷ but it is not known whether the coupling of ACh to the receptor results in the opening of transmembrane channels, the activation of ionic carrier mechanisms or some other mechanism that modifies mem-

brane permeability. Little is known about the ionic permeability changes associated with the function of the single receptor. It has been reported⁸ that crude acetylcholinesterase (AChE) preparations from electroplax contain material capable of inducing transient membrane permeability changes in lipid bilayers in response to ACh or carbachol, but the relationship to ACh receptor function is unclear.

We wish to report discrete changes of conductance after the incorporation of an ACh receptor preparation into artificial lipid bilayers. These events were related to a unit conductance that is probably associated with some fundamental function of the receptor.

Receptors were extracted by modification of published affinity chromatography techniques that make use of the curaric properties of neurotoxins from elapid snake venoms⁴⁻⁶. *Naja melanoleuca* d-toxin (10 mg) was attached to 15 ml of Agarose A-15 (Biorad) beads by the cyanogen bromide reaction⁹⁻¹¹ and a typical receptor extraction then proceeded as follows. Twenty-five whole mouse brains were homogenised in 10 volumes of 0.05 M sodium phosphate buffer, pH 7.4, containing 0.1% Triton X-100 detergent. The particulate matter was removed by centrifugation at 10,000g for 30 min and the supernatant was passed through the toxin-Agarose gel. The gel was then washed with the same buffer-detergent solution plus 1 M NaCl to disassociate material bound by nonspecific ionic interactions, followed by receptor elution with a 0.0 to 0.5 M carbachol gradient in buffer-detergent. Protein determinations¹² indicated the possible presence of two protein peaks following exhaustive dialysis against 0.05 M phosphate buffer.

The ability of the original fractions from peak 1 (containing about 0.2 M carbachol from the gradient) to affect ionic permeability were examined in lipid bilayers as described earlier¹³. This involved a dilution of the original fractions by 400:1 to at most 25 ng ml⁻¹ of total protein and 5×10^{-4} M of carbachol in 100 mM NaCl on one side (*cis*) of the bilayer. The other side (*trans*) contained 1 M NaCl and the gradient thus formed allowed both sodium and chloride conductances to be estimated separately, depending on the polarity of the voltage clamp across the membrane. The chamber was made of a fluorochlorocarbon material (Kel-F) and contained a 1.4-mm hole over which the bilayer was spread. A Keithley 427 current amplifier was used to amplify the current changes sufficiently for detection on a strip-chart recorder. Clamp was maintained at + or -80 mV.

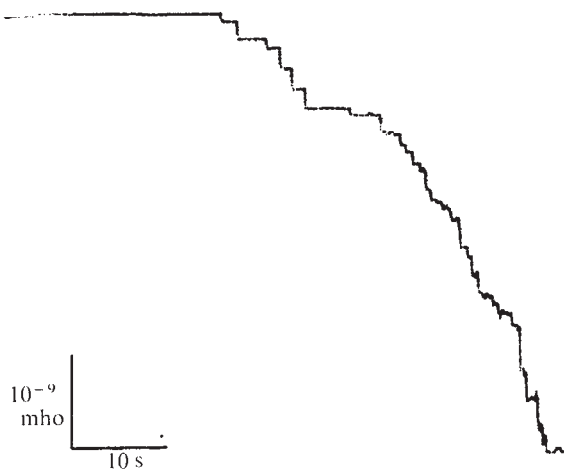


Fig. 1 Initial increase in chloride conductance after addition of receptor to *cis* side of bilayer. The final concentration of total protein was at most 25 ng ml⁻¹ with about 5×10^{-4} M carbachol.

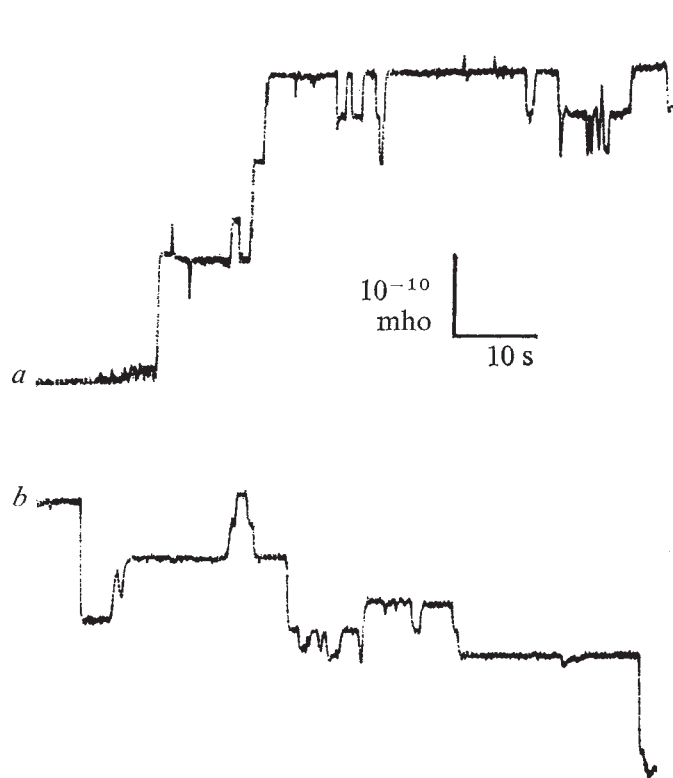


Fig. 2 The same receptor preparation as in Fig. 1 except that 2.5×10^{-3} M of tubocurarine was added to the *cis* side of the bilayer. The scale is at 10 times higher resolution with (a) the chloride conductance and (b) the sodium conductance.

Under these conditions, two sizes of conductance changes were electronically detected with step increases of 2.4×10^{-10} mho (Cl^-) and 1.5×10^{-10} mho (Na^+) for large quanta and 5.9×10^{-11} mho (Cl^-) and 3.7×10^{-11} mho (Na^+) for small quanta. The initial conductance increase was monotonic and the chloride conductance recording is shown in Fig. 1. Addition of tubocurarine at competitive concentrations (2.5×10^{-3} M) seemed to slow the rate of occurrence of large quanta by about 30:1 and the smaller quanta became prominent (Fig. 2a (Cl^-) and b (Na^+)). A similar effect seemed to occur with addition of similar concentrations of atropine, although the nature of the studies made quantitation difficult.

When the carbachol was removed by dialysis of the receptor preparation against phosphate-detergent solution, the rate of occurrence of large quanta appeared to be reduced relative to the occurrence of small quanta. When the receptor was dialysed against phosphate alone, however, activity ceased, indicating that detergent was necessary to preserve the electrical properties of the molecular entity involved. In addition, if the receptor was eluted by a carbachol gradient in phosphate but in the absence of detergent, no electrical activity was detected when the preparation was incorporated into the bilayer. This occurred in spite of the detection of similar elution peaks. We had previously found that Triton X-100 alone did not affect our bilayer system at the concentrations used. Gel electrophoresis demonstrated the presence of one primary protein peak, although the possibility that the electrical activity is caused by another protein component cannot be excluded. Peak 2 showed qualitatively similar electrical activity.

The ionic selectivities of the large and small quanta and their relative size suggests that the large quanta represent an aggregate of four of the small quanta. This might be

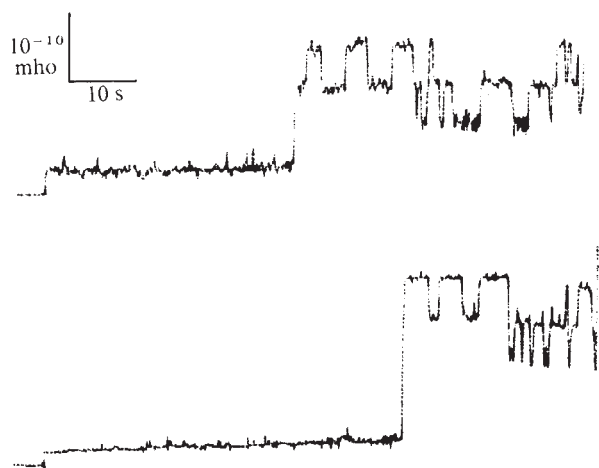


Fig. 3 Conductance changes (Cl^-) for two runs after the receptor had been dialysed against 0.05 M phosphate buffer, pH 7.4, containing 0.1% Triton X-100 for 72 h with six changes of dialysate.

related to theories that the receptor can exist as a tetramer^{14,15}. We note that the electrical activity observed is consistent with the theory that Ach-mediated membrane permeability changes might occur by ionic channel gating.

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effects of morphine on cholinergic transmission. On the only central neurones known to receive cholinergic endings, the Renshaw cells⁵, morphine administered electrophoretically from micropipettes has been shown to modify cell activity in several ways; to reduce the depressant action of glycine⁶, to increase the latency of action potentials evoked by a ventral root stimulus⁷ and to excite⁷.

To assess the relevance of these effects to the opiate syndrome⁸, we performed experiments with naloxone, a narcotic antagonist essentially devoid of agonist activity⁹. As a further test of specificity of effects observed with morphine, three morphinans were also studied: levorphanol, an active opiate; dextrorphan, an isomer devoid of narcotic activity; and levallorphan, a narcotic antagonist. The results indicate that excitation at nicotinic receptors for acetylcholine may be of relevance to the opiate syndrome.

Experiments were performed on 23 cats anaesthetised with pentobarbitone sodium. The spinal cord was prepared for stimulation and recording using conventional techniques¹⁰. Renshaw cells were located by the responses to stimulation of ventral roots. Extracellular recordings were obtained with the centre barrels of seven barrel micropipettes and the outer barrels contained the drugs to be ejected electrophoretically. These were: morphine SO_4 (70 mM), naloxone HCl (0.1 M), levorphanol tartrate (0.1 M and 50 mM in 100 mM NaCl), dextrorphan tartrate (0.1 M), levallorphan tartrate (0.1 M), acetylcholine Br (0.5 M), L-glutamate Na (0.5 M), L-aspartate Na (0.5 M), DL-homocysteate Na (0.2 M), glycine (0.5 M pH 3), γ -aminobutyric acid (0.5 M pH 3), atropine SO_4 (10 mM in 165 mM NaCl), dihydro- β -erythroidine HBr (10 mM in 165 mM NaCl), nicotine HCl (0.2 M), acetyl- β -methylcholine Cl (0.5 M).

Morphine (30-80 nA) excited 42 of 55 Renshaw cells tested. This effect was readily demonstrated on cells excited by low concentrations of acetylcholine (ejecting current less than 10 nA) and was not necessarily accompanied by bursts of firing. With cells relatively insensitive to acetylcholine, ejection of morphine with relatively high currents was more likely to produce bursts of action potentials with little rise in mean firing rate. Whereas the ejecting current of morphine required to produce cell firing was approximately ten times that of acetylcholine, much lower currents of morphine enhanced the excitant action of acetylcholine and/or an excitant amino acid. Excitation of a Renshaw cell by morphine acetylcholine and DL-homocysteate is shown in Fig. 1.

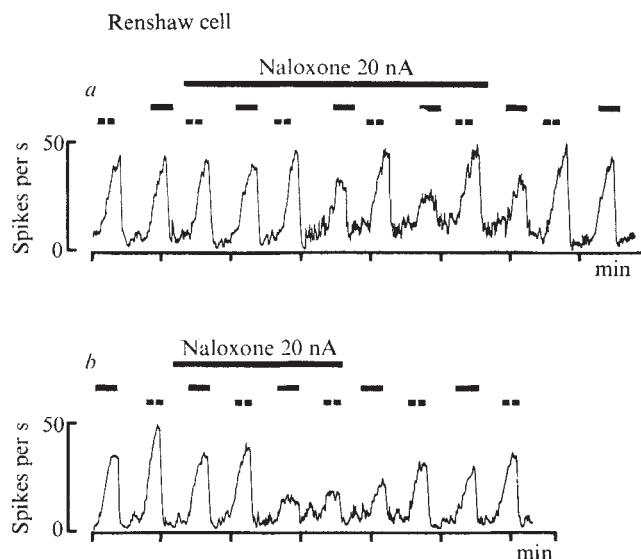


Fig. 1 The effect of naloxone on excitation of a Renshaw cell by, a, morphine (30 nA) (—) and DL-homocysteate (30 nA) (---) and, b, by morphine (30 nA) (—) and acetylcholine (7 nA) (---). The times of electrophoretic ejection of compounds are marked by horizontal bars above each record of cell firing.

Opiate agonist-antagonist effects on Renshaw cells and spinal interneurons

ANALGESIC doses of morphine change the levels^{1,2} and release^{3,4} of acetylcholine from the central nervous system. At the single neurone level, however, little is known of the

Excitation by morphine was antagonised by naloxone. Ejecting currents of naloxone (10–30 nA) adequate to block the effects of morphine were usually without effect on cell firing but higher concentrations sometimes produced bursts of firing. Excitation by acetylcholine but not that by an amino acid was also reduced by naloxone. Figure 1 illustrates the differentiation between these two types of receptor by naloxone. Naloxone also reduced the excitant effect of nicotine but not that of acetyl- β -methylcholine on Renshaw cells, indicating a probable action at nicotinic receptors on these neurones. Dihydro- β -erythroidine (1–5 nA) readily reduced the action of acetylcholine on Renshaw cells and also reduced excitation by morphine. Atropine was a relatively poor antagonist of excitation of Renshaw cells by acetylcholine but again the action of morphine was also reduced in parallel with acetylcholine.

The other effects of morphine on Renshaw cells were not antagonised by naloxone. Naloxone acted like morphine in prolonging the latency of the initial action potentials of Renshaw cells in response to a ventral root stimulus but was without effect on the depressant action of glycine and GABA and did not modify the antagonism of glycine by morphine.

Morphine (5–100 nA) did not excite a sample of spinal interneurons located by firing in response to stimulation of peripheral nerves. Morphine (17 of 25 cells) and naloxone (10 of 12 cells) were depressants of these neurones and no antagonism of the action of morphine by naloxone was observed. This action of morphine has been previously reported¹¹ but the lack of antagonism by naloxone casts doubt on its relevance to the effects of systematically administered morphine.

All three morphinans tested reduced the sensitivity both to acetylcholine and an amino acid excitant of a proportion of Renshaw cells, an effect accompanied by a reduction in action potential amplitude. This was the only effect observed with dextrorphan (eight cells) and levallorphan (five cells) but with levorphanol, the active opiate, excitation was observed with five of twelve Renshaw cells. The reduction of effectiveness of all excitants is possibly a local anaesthetic effect¹² as these compounds are known to block conduction in squid axon¹³ and are more potent than morphine in this respect.

Concentration attained when drugs are administered micro-electrophoretically are unknown and therefore the relevance of effects observed with this method to those occurring after systemic administration can be difficult to assess. Nevertheless, as excitation by morphine at nicotinic receptors for acetylcholine was the only action of this alkaloid antagonised by naloxone the effect warrants further investigation. Studies of the distribution of opiate receptors in the brain of the rat based on the displacement of bound naloxone by active and inactive morphine-like compounds have found good correlation with the distribution of acetylcholine¹⁴. In this species electrophoretically administered morphine excited one half of the brain stem neurones sampled¹⁵ but no correlation with the effects of acetylcholine, noradrenaline or 5-hydroxytryptamine was observed.

Our experiments are being extended to cholinceptive cells in other regions of the central nervous system of the cat.

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Lithium and the monoamine neurotransmitters in the rat hippocampus

ALTHOUGH the psychoactive properties and therapeutic value of lithium have long been recognised¹, relatively little is known about its mode of action in the central nervous system². This is partly due to the lack of a complete understanding of the biological basis of mental disorders and to the variety of biological functions that can be affected by lithium.

The putative monoamine neurotransmitters, noradrenaline, dopamine and serotonin have been implicated in such functions, and indeed lithium influences their metabolism in the brain^{3,4}. The precise distribution of monoamine-containing cell bodies, fibres and synaptic terminals has been well documented for several targets within the brain. Recently, the monosynaptic pathway between the catecholamine-containing pontine nucleus locus coeruleus (LC) and the hippocampus was studied physiologically and pharmacologically^{5,7}. In this pathway the iontophoretic application of noradrenaline suppresses spontaneous discharges of hippocampal pyramidal cells⁶ as does stimulation of the LC (ref. 7). These noradrenergic synapses in the hippocampus thus provide an ideal test system for the search for the mechanisms of psychoactive drugs in the nervous system. I describe here an attempt to elucidate a possible mode of action of lithium in the hippocampus. This was done by searching for possible interactions between the effects of iontophoretically applied lithium and several putative neurotransmitters as well as the electrical stimulation of the LC, on firing rates of hippocampal pyramidal cells.

I used male Zivic Miller rats (150–200 g) anaesthetised with halothane. Methods of recording and drug iontophoresis have been described elsewhere⁸. Briefly, spike discharges were recorded through the central barrel of a five-barrel micropipette. Three of the outer barrels contained lithium chloride (Li^+ , 0.1–0.2 M); acetylcholine chloride (ACh, Merck, 2.5 M); L-noradrenaline-HCl (Aldrich, 1 M); serotonin creatinine sulphate (Aldrich, 0.5 M) and γ -aminobutyric acid (GABA, Aldrich, 1 M). Each compound was ejected as cations, and retained with a negative holding current. The fourth outer barrel was filled with 3 M NaCl and ejected a continuous balancing current⁸. Stimulating electrodes were placed in the LC as described previously⁷.

The activity of thirty-five cells in the pyramidal cell layer of the dorsal hippocampus was monitored in eight rats. When applied iontophoretically (with a current range of

25–100 nA) Li^+ did not have a consistent direct effect on firing of hippocampal cells. Only three of the cells tested were slightly excited (120–140% of predrug rate) and two were slightly slowed (Fig. 1a), whereas the firing patterns or spike sizes of thirty other cells were not affected. Noradrenaline had a consistent and long lasting inhibitory action on spontaneous and Ach-enhanced hippocampal activity⁶. All fifteen cells tested were inhibited during application of noradrenaline (current range 50–100 nA applied in pulses of 15 s). In seven of the cells inhibition was reversibly antagonised during the concurrent application of Li^+ (Fig. 1b). In five more cases Li^+ (in 1.0 M concentration within the pipette) antagonised the cellular response to noradrenaline but no complete recovery was demonstrated even 10–30 min after application of Li^+ . Only three cells were unaffected by Li^+ .

The interaction between Li^+ and serotonin was tested in seven cells. Like noradrenaline, it was inhibitory with respect to spontaneous hippocampal cellular activity. Concurrent application of Li^+ for 2–3 min reversibly antagonised the inhibitory responses of three cells to serotonin. Four other cells were unaffected (Fig. 1a).

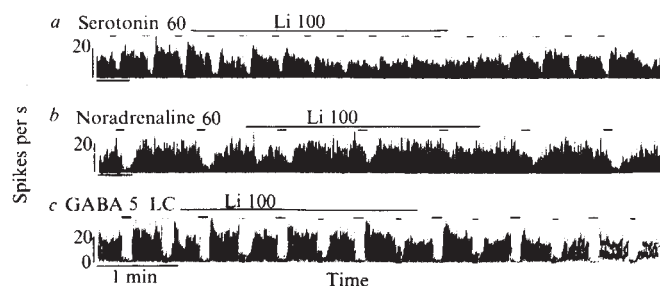


Fig. 1 Interactions between iontophoretic application of Li^+ , several putative neurotransmitters and LC stimulation. Hippocampal cellular activity in the pyramidal layer was integrated over 1-s intervals and recorded on an ink recorder. *a*, The effects of Li^+ on the responses to serotonin applied with an injection current of 60 nA at fixed regular intervals (bars). The concurrent application of Li^+ (with current of 100 nA), for 2–3 min caused a slowing of the spontaneous firing, but also blocked the response to serotonin. This response was reinstated 1–2 min after termination of Li^+ current. *b*, Same as in (*a*), with a different cell, Li^+ antagonised the response to noradrenaline with no noticeable effect on spontaneous activity. *c*, Li^+ effects tested against the iontophoretic application of GABA and stimulation of LC. Stimulation parameters: pulses of 0.1 ms, 0.4 nA, applied through a concentric stimulating electrode at a rate of 10 per s for 5 s. Li^+ had no effect on the response to GABA but there was an antagonism of the response to LC stimulation. Note the difference in time scale between (*a*), (*b*) and (*c*).

Among the other compounds tested, GABA exerted the most potent inhibitory action towards hippocampal activity and its effect was pronounced even with small ejection current (0–5 nA). But none of the responses of fifteen cells tested was affected by the iontophoretic application of Li^+ in the current ranges that proved to be effective for antagonism of the responses to noradrenaline and serotonin (Fig. 1c). Similarly, acetylcholine, a putative neurotransmitter in the hippocampus had an excitatory action which was not modified by the iontophoresis of Li^+ . The absence of interaction was noted in a number of cells, although no systematic tests were made.

Finally, electrical stimulation of the LC, the source of hippocampal noradrenergic fibres and terminals produce noradrenaline-like inhibition⁷ (Fig. 1c). In five out of six cells tested, LC stimulation was at least partially antagonised by concurrent iontophoretic application of Li^+ . This antagonism did not result, however, in a complete elimination of the response to LC stimulation. Since at least some of the

noradrenergic terminals on the recorded cell are probably remote from the pipette tip and may not be affected by the Li^+ ejected, a complete antagonism could be difficult to achieve.

Under the conditions of these iontophoretic experiments, there was a built-in control for defining the specificity of the effects of Li^+ . When Li^+ or the other compounds were not being ejected, they were retained in the pipettes with a continuous negative current. To neutralise the continuous retaining currents at the pipette tip between and during drug ejection, positive current, that is, Na^+ ions, was continuously ejected from the balance barrel. Therefore, in all cases, the effects of Li^+ were actually tested against the effects of Na^+ .

Lithium can exert its antagonistic action by changing the ionic balance in the recorded cell environment. Although this is considered to be a possible effect of Li^+ in living tissue^{8,10}, it is not likely to be the cause for the specific effect demonstrated here since spontaneous firing as well as GABA-induced inhibition were not modified in the recorded hippocampal cells. A more likely alternative mechanism for modification by Li^+ is the cyclic AMP system. It has been postulated that cyclic AMP mediates the electrophysiological response to noradrenaline in the rat cerebellum¹¹ and there is some evidence for such a case in rat hippocampus, where the phosphodiesterase inhibitor, papaverine, potentiates the response of hippocampal cells to noradrenaline applied iontophoretically, and prostaglandin E_1 antagonises this response⁶. Lithium has also been found to block noradrenaline-induced formation of cyclic AMP in rat cortical slices¹² with relatively low concentrations, and this, together with the data reported here supports the hypothesis that cyclic AMP mediates monoamine response in the rat hippocampus.

Although my data are compatible with the view that Li^+ could block the noradrenaline receptor through an action on cyclic AMP synthesis, earlier alternative assumptions of Li^+ action based on such findings as increased uptake of noradrenaline by brain synaptosomes¹³, decrease in the release of noradrenaline and serotonin from brain slices¹⁴, cannot be eliminated. The iontophoretic mode of application, in spite of its many advantages, tests actually the acute effects of Li^+ which might be different from the effects of a chronic Li^+ treatment. Further electrophysiological study of Li^+ actions in a chronically treated preparation are required to clarify these possibilities.

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Suppression of phage nonsense and temperature-sensitive mutants by an *suA* mutant *E. coli*

BECKWITH and Scaife^{1,2} showed that the suppressor allele *suA* of *Escherichia coli* does not suppress nonsense codons in the lactose operon, but does relieve the polarity effect of UAG, UAA and possibly frameshift mutations. It is shown here that *suA E. coli* strongly suppresses phage T4 amber mutations and weakly suppresses phage T4 temperature-sensitive and amber

Table 1 Suppression of T4 amber mutants by the *suA* allele, expressed in burst size

	CR63*	<i>suA</i> *	<i>su</i> †	<i>E. coli</i> B†
	Catalytic functions			
<i>amNG83</i> (30)	46	77	0.01	0.003
<i>amE1017</i> (30)	70	131	0.03	0.008
<i>amNG352</i> (42)	21	20	0.06	0.04
<i>amE93</i> (42)	32	22	0.0	0.01
<i>amB22</i> (43)	86	339	0.01	0.02
<i>am4301</i> (43)	83	189	0.06	0.01
<i>amB3</i> (46)	14	112	0.1	0.01
<i>amNG371</i> (46)	55	263	0.7	0.08
<i>amNG106</i> (47)	77	256	0.2	0.06
	Stoichiometric functions			
<i>amN50</i> (20)	108	5	0.0	0.005
<i>amB17</i> (23)	62	102	0.06	0.01
<i>amB272</i> (23)	51	18	0.15	0.01
<i>amN65</i> (24)	66	2	0.0	0.01
<i>amB26</i> (24)	79	4	0.06	0.01
<i>amA453</i> (32)	56	25	0.03	0.06
<i>amE1</i> (36)	10	7	0.03	0.01
<i>amC15</i> (36)	63	5	0.06	0.03
<i>amN52</i> (37)	61	152	0.01	0.06
<i>amB280</i> (37)	37	1	0.5	0.08
T4D	143	265	47	226

Phage infections were carried out at 26.6° C. After each amber mutant designation the gene in which the mutant is defective is indicated in parentheses.

* Average of two experiments.

† Average of three experiments.

mutations. Suppression of the inhibitory effect of chloramphenicol on phage T4 growth by *suA* was also shown. In addition, amber mutants of phage T7 were observed to be suppressed by *suA*. These results suggest that *suA* in addition to suppressing polarity in *E. coli* also increases translational ambiguity in T4 and T7-infected cells, especially for the amber codon.

All phage T4 strains used in this study as well as *E. coli* strains CR63 (*su*-1+ UAG), *E. coli* B (*su*-) and *E. coli* BB (containing an amber suppressor, as described by Wilson and Kells³) were from the California Institute of Technology stock collection, under the case of W. Wood. *E. coli* CAJ68 (containing a UGA amber suppressor) was provided by D. Mount, University of Arizona. *E. coli suA* XA7007, F⁻, Δ (Lac-Pro), Mal⁻, B₁⁻, Str^r, Δλ and *E. coli su*- Sy106 were a gift from W. Summers, Yale University and were the strains used in all burst size determinations. Sy106 is an isolate of the original strain from which XA7007 was derived and has the same genetic markers. The presence of the identifying markers in *E. coli suA* XA7007 and *su*- Sy106 were confirmed here. A related *su*- strain, *E. coli su*- XA7014, was obtained from J. Beckwith. The phage T7 strains used were originally from the laboratory of W. Studier and were supplied by T. North at the University of Arizona.

The suppression of phage amber mutants (Table 1) was compared by burst size measurement in four *E. coli* strains (CR63, *suA*, *su*- and B). Unadsorbed phage, measured in a chloroform-treated aliquot taken before the end of the eclipse period, were subtracted when they constituted more than 5% of the final phage yield. Clearly *suA* is comparable with CR63 in the efficiency of its suppression of amber mutants whereas *su*- is about as inefficient as B. In general, amber mutants defective in catalytic functions showed greater burst sizes on suppression by *suA* than those defective in stoichiometric functions⁴. When further tested on plates, the efficiency of suppression by *suA* of most of the amber mutants was high enough to allow these mutants to form plaques. When either the *su*- strain obtained from Summers (Sy106) or the *su*- strain obtained from Beckwith (XA7014) was used as an indicator, no plaques were formed.

The suppression of phage temperature-sensitive (*ts*) mutants is compared in the *suA* and *su*- strains at various temperatures (Table 2). The *ts* mutants defective in catalytic functions seem to be significantly suppressed by *suA* at higher temperatures. Although there is some suppression of *ts* mutants defective in

Table 2 Suppression of T4 *ts* mutants by the *suA* allele, expressed in burst size

	25.6°C	28.0°C	30.0°C	34.0°C	38.0°C*	39.6°C	41.5°C	42.0°C†
	Catalytic functions							
<i>tsL88</i> (43) <i>suA</i>	200	336	290	407	93	55	14	2.1
<i>tsL88</i> (43) <i>su</i> -		226		370	36	29	0.02	0.087
<i>tsL91</i> (43) <i>suA</i>	43	129	107	65	25	61	27	2.1
<i>tsL91</i> (43) <i>su</i> -		126		179	26	8.4	0.5	0.506
<i>tsL109</i> (46) <i>suA</i>	227	335	436	305	119	46	6.0	1.1
<i>tsL109</i> (46) <i>su</i> -		199		378	21	8.1	0.5	0.622
<i>tsB10</i> (47) <i>suA</i>	192	419	488	326	101	4.2	4.0	5.3
<i>tsB10</i> (47) <i>su</i> -		79		16	2	0.8	0.1	0.531
<i>tsA52</i> (47) <i>suA</i>	258	390	470	385	90	26	5.0	6.6
<i>tsA52</i> (47) <i>su</i> -		190		181	8	4.0	0.3	0.436
	Stoichiometric functions							
<i>tsL65</i> (23) <i>suA</i>	147	370	567	388	51	1.7	0.04	1.0
<i>tsL65</i> (23) <i>su</i> -		177		477	50	6.6	0.005	0.001
<i>tsN29</i> (24) <i>suA</i>	178	435	514	438	3.1	0.09	0.04	0.8
<i>tsN29</i> (24) <i>su</i> -		184		297	1.2	—	0.002	0.002
<i>tsA4</i> (37) <i>suA</i>	198	301	887	534	131	142	158	24
<i>tsA4</i> (37) <i>su</i> -		54		235	35	27	18	34
<i>tsA31</i> (37) <i>suA</i>	192	284	367	335	98	126	125	37
<i>tsA31</i> (37) <i>su</i> -		117		123	43	61	31	56
T4D <i>suA</i>	151	185	380	262	138	160	389	121
T4D <i>su</i> -		287		351	125	125	108	144

After each *ts* mutant designation the gene in which the mutant is defective is indicated, as well as the *E. coli* host infected (either *suA* or *su*-).

*Average of two experiments.

†Average of three experiments.

stoichiometric functions by *suA* at higher temperatures, it is less striking.

The suppression of the phage amber mutants by CAJ68, *suA* and *su⁻* is shown in Table 3. *SuA* seems to be a significantly more effective host in supporting growth of amber mutants than *su⁻*, but not nearly as effective as CAJ68, which contains a specific amber suppressor. This low efficiency of suppression by *suA* of amber mutants is similar to the low efficiency of suppression of *ts* mutants reported above and in contrast to the high efficiency found for amber mutants.

Table 3 Suppression of T4 amber (*um*) mutants by the *suA* allele, expressed in burst size

	CAJ68*	<i>suA</i> †	<i>su⁻</i> †
Catalytic functions			
<i>umC166</i> (56)	265	2.1	0.0
<i>umC123</i> (47)	391	6.5	0.1
<i>umC102</i> (42)	131	0.1	0.0
Stoichiometric functions			
<i>umC23</i> (37)	296	0.7	0.05
<i>umC105</i> (34)	361	3.0	0.0
T4D	245	337	308

Phage infections were carried out at 26.6° C. After each amber mutant designation the gene in which the mutant is defective is indicated in parentheses.

* Average of two experiments.

† Average of three experiments.

The burst sizes of phage T7 amber mutants in three *E. coli* strains (BB, *suA* and *su⁻*) are compared in Table 4. *E. coli suA* is comparable with *E. coli* BB (which contains an amber suppressor) in the efficiency of its suppression of T7 amber mutants. In contrast, growth of T7 on *E. coli su⁻* is at least an order of magnitude less efficient. Where *E. coli su⁻* was used as a plating indicator, no plaques were obtained. When *E. coli* BB or *suA* was used as a plating indicator, large distinct plaques were formed. *E. coli suA* thus seems to suppress T7 and T4 amber mutants with about the same efficiency.

In the experiments described in Table 5, chloramphenicol was added 1.5 min after infection. At 11.5 min after infection it was diluted out so that phage growth could proceed. Recovery from the chloramphenicol effect was then measured in terms of burst size after completion of the infectious cycle. As Table 5 shows, burst sizes after chloramphenicol treatment were only 22–27% of normal in *E. coli* CR63, S/6, and *su⁻*. However in *E. coli suA* the burst size after chloramphenicol treatment was 70% of normal.

Table 4 Suppression of T7 amber mutants by the *suA* allele, expressed in burst size

	<i>E. coli</i> BB*	<i>suA</i> †	<i>su⁻</i> †
<i>am28</i> (5)	141	178	15‡
<i>am29</i> (3)	137	124	22‡
T7 wild type	142	80	98

Phage infections were carried out at 30° C. After each amber mutant designation the gene in which the mutant is defective is indicated in parentheses.

* Average of three experiments.

† Average of two experiments.

‡ These apparent burst sizes are probably due to unadsorbed phage.

The results presented here indicate that the *E. coli suA* mutant suppresses amber mutations of phages T4 and T7, *ts* and amber mutations of phage T4, as well as the chloramphenicol-induced inhibition of phage T4 growth. The most plausible explanation

for the suppression of such varied blocks to growth is that the *suA* mutation of *E. coli*, when present in phage T4 and T7-infected cells, increases the level of translational ambiguity. In its ability to suppress various different mutations, the *suA* allele seems similar to ram, the ribosomal ambiguity mutation of *E. coli*⁵. It may be that *suA* is also altered in a translational element. The product of the *suA* gene has been partially purified by Wetekamp and Ehring⁶ and has been shown to act at translation even when uncoupled from transcription.

Suppression of amber, *ts* and amber mutants defective in stoichiometric functions was usually inefficient compared with suppression of such mutants defective in catalytic functions (Tables 1, 2 and 3). Catalytically acting proteins may allow normal burst sizes even when present in reduced amounts whereas stoichiometrically acting proteins will not allow normal burst sizes unless present at near wild-type levels⁴. The burst sizes obtained after suppression of amber, amber and *ts* mutations in stoichiometric functions implies that suppression by *suA* usually restores a low level of functional gene product. The few stoichiometric mutants which were suppressed efficiently suggests that the level of suppression is context or gene specific.

Table 4 indicates that *suA* is an effective suppressor of phage T7 amber mutations, when burst size is the criterion of suppression. Summers⁷ using the *suA* strain used here (XA7007) observed that on infection by a phage T7 amber mutant defective in gene 1 (RNA polymerase) mRNA formation continued beyond the amber codon, whereas in *E. coli su⁻* it did not. Furthermore, he noted in the *suA* infection that short polypeptide fragments corresponding to a defective gene 1 product were formed. This indicates that at least some of the time translation is terminated at the amber codon in the *suA* host. However his result does not preclude the possibility that occasionally synthesis is continued beyond the amber codon. As discussed above, under conditions where essential enzymes are produced at a low level, phage yield may nevertheless be normal because of the catalytic nature of enzyme function. Thus Summers' results are compatible with our conclusion that *suA* is an effective suppressor of the amber phenotype of phage T7.

The effect of the *suA* allele on suppression of the amber codon as observed in phage T4 and T7-infected cells was not observed in uninfected *E. coli*^{1,2,8}, although only few bacterial mutants were tested. Hsu and Weiss⁹ and Schedl *et al.*¹⁰ have presented evidence that ribosomes extracted from uninfected cells are more active than ribosomes extracted from T4-infected cells *in vitro* when used in translating mRNA from *E. coli* or MS2. This suggests that T4 proteins alter ribosomal template specificity. Smith and Haselkorn¹¹ showed that proteins either induced or modified by phage T4 become associated with the *E. coli* ribosomes after T4 infection. Similar proteins may be produced by phage T7. It may be that one or more of these proteins alter the ribosomes' susceptibility to the effect of the host *suA* allele. If the *suA* allele acts at the level of the ribosome, which is then the initially determined phenotype of the mutant, suppression of polarity^{1,2} might be due to an increased tendency of *suA* cell ribosomes to continue along the mRNA after encountering a nonsense codon so as to be available at the next initiating codon. The continuation of the ribosomes would also protect the mRNA against the degradative action of RNases.

Morse¹² presented evidence that chloramphenicol induces an

Table 5 Suppression by the *suA* allele of the chloramphenicol-induced inhibition of phage T4D growth, expressed in burst size

	CR63	S/6	<i>su⁻</i>	<i>suA</i>
T4D, no chloramphenicol	272	170	116	130
T4D, with chloramphenicol	60	43	31	90
Burst size as % of normal burst	22%	26%	27%	70%

Phage infections were carried out at 30° C. To 0.5 ml of bacteria at about 2×10^8 cells ml⁻¹, 0.5 ml of phage were added to give a multiplicity of infection of 13 phage per bacterium. When the infection had proceeded for 1.5 min, 100 µg of chloramphenicol in 0.1 ml was added. At 11.5 min after infection, 0.1 ml of culture was diluted 4×10^{-4} -fold and growth was allowed to occur for 2 h. At this time phage progeny were determined by plating on *E. coli* CR63 and the burst sizes calculated. All figures are the average of three experiments.

artificial polarity effect on distal mRNA in the *E. coli* tryptophan operon, and that this polarity effect is relieved by the *suA* allele. Table 5 indicates that *suA* also suppresses the effects of chloramphenicol on phage growth. Thus there are somewhat parallel effects of *suA* with respect to chloramphenicol in the two systems. Chloramphenicol is thought to block the transfer of the amino acid from aminoacyl tRNA to the growing polypeptide chain¹³. Since chloramphenicol affects a process taking place on the ribosome it is reasonable to suppose that the *suA* reversal of chloramphenicol inhibition could be due to an effect of *suA* on ribosomal function.

Kuwano *et al.*¹⁴ found a greater release of mRNA fragments *in vitro* by a crude extract of *su*⁻ compared with a similar extract of *suA*. This release was thought to be brought about through the action of an endonuclease which they tentatively designated endonuclease A and which was presumed to be present in *su*⁻ cells, but defective in *suA* cells. Although there is no simple way to reconcile this result with the suppression effects I have observed, it could be supposed that endonuclease A binds to ribosomes and affects the accuracy of translation.

In conclusion, the observations reported here suggest that the *suA* allele produces an altered translational element which enhances mistranslation of the genetic code in phage T4 and T7-infected cells. The wild type form of the *suA* translational element may be necessary both for accurate translation and the dislodgement of the ribosome from the mRNA after reading a nonsense codon.

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Correlations between plasma ACTH concentrations and breathing movements in foetal sheep

BREATHING movements *in utero* are a normal accompaniment of foetal life¹⁻³. Dawes *et al.*³ reported an instance of reduced foetal breathing in a sheep with spontaneously occurring foetal hypoxaemia. Subsequent observations have shown similar reductions associated with foetal hypoglycaemia, infection, and maternally administered hypoxaemia. In addition they are influenced by hypercapnia, temperature

and time of day⁴. In human pregnancy reduced foetal breathing is associated with maternal hypertension and foetal growth retardation⁵. Thus breathing movements have been used as an index of foetal health. The concentration of adrenocorticotrophic hormone (ACTH) in the plasma of foetal sheep increases during hypoxaemia, haemorrhage and catecholamine infusion (refs 6-8 and C. T. J., K. B., J. G. Ratcliffe and J. S. R., unpublished observations) and may therefore reflect foetal condition. At present blood gas values, pH and heart rate are used as indices of foetal health *in utero* in animals and man. But we have observed a wide variation in plasma ACTH concentration and foetal breathing movements in foetal sheep *in utero* in circumstances in which blood gas values, arterial pH and heart rate were in the normal range. The present report describes a close correlation between the amount of foetal breathing and the foetal plasma ACTH concentration.

In 18 sheep of mixed breeds 120-145 d pregnant with catheters previously implanted in the foetal trachea and carotid artery, carotid blood samples were taken from

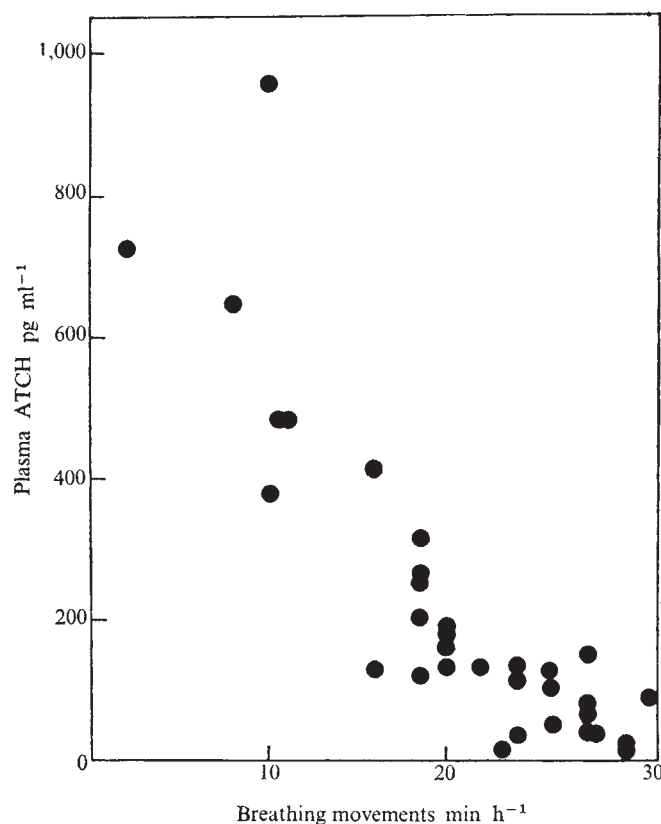


Fig. 1 Foetal carotid plasma ACTH concentration and incidence of foetal breathing movements. Each point, represents the foetal plasma ACTH concentration either at the beginning or the end of the hour that the incidence of foetal breathing was measured. Observations at both times were made on most animals.

mother and foetus and ACTH and corticosteroid concentrations were measured as previously described^{3,6}. Sampling took place at least 1 week after surgery, between 9000 a.m. and 1030 a.m. The mean foetal carotid arterial pH and P_{O_2} were respectively 7.37 ± 0.07 (s.e.m.) and 22 ± 0.5 mm Hg and the mean foetal heart rate was 157 ± 14 beats min^{-1} . There was a reciprocal correlation between the incidence of foetal breathing movements over an hour and the foetal plasma ACTH concentration at the beginning and end of that period. There was no correlation with foetal blood gas values, pH, plasma corticosteroid concentration or heart rate. The relationship observed between foetal breathing and plasma ACTH concentration was not directly related to gestational age.

In the foetal sheep dissociation of the electrocorticogram

into high and low voltage activity is first seen around 120 d gestation. Foetal breathing movements are only seen in association with the low voltage activity characteristic of rapid eye movement sleep³. Bernhard and coworkers⁹ have suggested in the foetal sheep that the development of the dissociation and hence rapid eye movement sleep probably originates in the corpus callosum and thalamus. It is possible that the activity in these structures during rapid eye movement sleep may have some connection with that which influences the hypothalamic control of ACTH secretion.

The inverse correlation between foetal breathing movements and ACTH concentrations is not surprising since both are influenced, in opposite directions, by hypoglycaemia, hypoxia, haemorrhage and infection (refs 4, 5, 8, 10–12; K. B., G. S. Dawes and J. S. R., unpublished observations). But none of these factors was directly responsible for the variation in breathing movements or plasma ACTH concentration in the present series of observations. The wide variation in ACTH concentration observed in the foetus and its sensitivity to various types of stimulation such as hypoxaemia or catecholamine infusion^{6,7,10} is surprising in view of the relative insensitivity to ACTH of the foetal adrenal output of glucocorticoids^{6,13}. This explains the absence of a correlation between breathing movements and plasma corticosteroid concentration in foetal sheep.

These observations indicate that the measurement of foetal blood gas values, arterial pH and heart rate alone do not fully reflect foetal condition. Other factors such as the incidence of foetal breathing movements and plasma hormone concentrations are required for the assessment of the physiological state of the foetus *in utero*.

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Redistribution of endogenous gibberellins in geotropically stimulated roots

THE difference in growth rate of the two sides of geotropically reacting plant organs is generally explained by the well documented unequal auxin concentrations in the two halves. The auxin ratio in horizontally placed stems, coleoptiles, and roots usually attains a value of about 2:1 in favour of the lower half.

But, Phillips¹ and Railton and Phillips² have shown that the ratio of gibberellin activity in diffusates from opposite halves of geotropically reacting green *Helianthus* hypocotyls and etiolated *Zea* coleoptiles reaches values of about 9:1 and 4:1, respectively, in favour of the lower (convex) half. These findings raise the question of the possible role of gibberellins in the geotropic reactions of these organs.

In horizontally positioned main roots, auxin accumulation in the lower (concave) half, presumably in supraoptimal concentrations, has been postulated to retard the elongation of that half, thus bringing forth the positive geotropic curvature. The correctness of this view has been questioned for some time^{3,4}, and unspecified inhibitors have been suggested as mediators of the differential rates of elongation^{5–8}. In this situation, and in view of the reports on asymmetrical gibberellin distribution in geotropically stimulated shoots, it would be interesting to determine the distribution of gibberellins in horizontally positioned roots. For this purpose the following experiments were carried out.

Seeds of *Vicia faba*, cultivar Erfordia, were soaked for 4 h in tap water and sown in moist vermiculite in the dark at 25° C. All subsequent operations took place under a green safelight. After 4 d, 500 (or 250) seedlings having roots approximately 15 mm long were selected for root uniformity. Of these, 250 (or 125) were left upright and 250 (or 125) were kept horizontal for 30 min. Using a sharp blade, each root was then split lengthwise into two halves. Four batches of root halves were thus obtained: upper and lower halves of horizontally oriented roots, and left and right halves of upright roots. Each batch was extracted at 2° to 3° C with three changes of 80% methanol over 36 h. The acidic fractions soluble in ethyl acetate were passed through a column of polyvinyl pyrrolidone (PVP), eluted with phosphate buffer (pH = 8.0), and transferred in diethyl ether to small volumes of ethanol. These were loaded on silica gel thin layer chromatography plates, which had been prerun in a 98:2 (v/v) mixture of ethanol and acetic acid⁹ and activated at 105° C for 1 h. The plates were developed with isopropanol, 25% specific gravity 0.91 ammonia and water, 10:1:1 (v/v). The chromatograms were divided into 10 transverse strips each of which was scraped off, eluted with 2 ml distilled water, and bioassayed for gibberellin activity in the *Lactuca* hypocotyl assay¹⁰.

As a check on the accuracy of the splitting of the roots, the dry weights of matching, extracted halves (250 in each group) were compared. They were: upright roots, left halves: 0.54 g, right halves: 0.56 g; horizontal roots, upper halves: 0.42 g; lower halves: 0.43 g.

Our results confirm the few available reports on the occurrence of gibberellins or gibberellin activity in roots^{11–13}. As shown in Fig. 1, more gibberellin activity was found on chromatograms of extracts from the upper than from the lower halves of horizontally oriented roots, whereas the amounts of gibberellin activity detected in left and right halves of upright roots were nearly identical.

By comparison with gibberellic acid (GA₃) standards the gibberellin activities in the upper and lower halves of horizontal roots were found equivalent to 31.0 and 17.5 ng GA₃ per g dry weight of tissue, respectively, in experiment *b* (Fig. 1). In experiment *c*, representing a different set of roots, the figures were 35.0 and 9.5 ng, but the growth

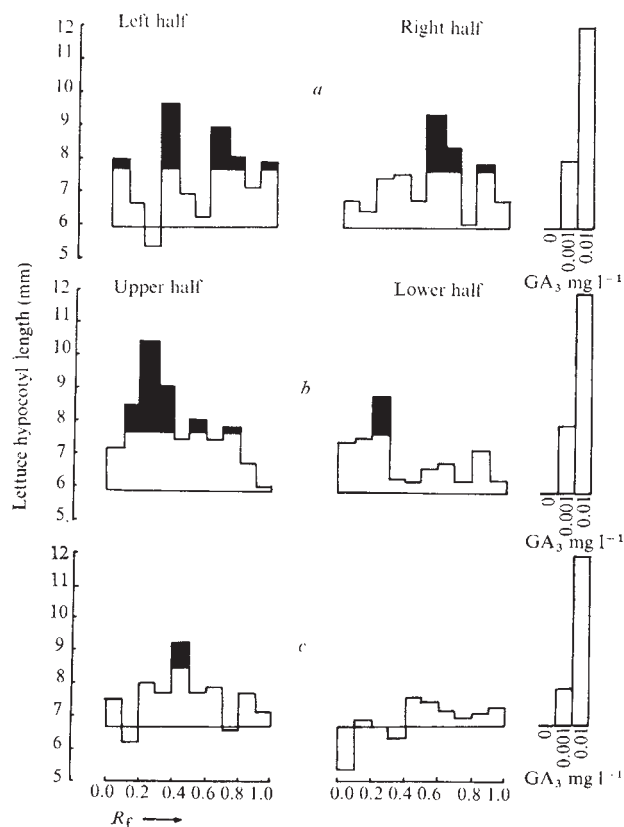


Fig. 1 *Lactuca* hypocotyl bioassays of acid, ethyl acetate-soluble fractions of chromatographed extracts of halved *a*, upright and *b* and *c*, horizontal *Vicia faba* roots. Extract equivalent to 0.2 g dry weight of roots in *a* and *b*, and to 0.1 g dry weight in *c*. A difference of 1.89 mm between any two readings is needed for statistical significance at the 5% level (base of the black parts). Gibberellic acid (GA_3) at 0.001 mg l^{-1} equals $2.6 \times 10^{-9} \text{ M}$.

promotions by the extract of the lower halves were too small to reach statistical significance. (The GA_3 ratio in favour of the upper half was 1.8:1 in experiment *b*; and in experiment *c* the calculated value was 3.7:1). In upright roots (*a*) the GA_3 activities in opposite halves were equivalent to 26 and 24 ng GA_3 per g dry weight.

In further experiments, to be reported in detail elsewhere, zones of the chromatograms running at the same R_f s as GA_3 were eluted with ethanol, methylated¹⁴ and subjected to gas liquid chromatography. The methyl ester of GA_3 was identified at the position (retention time) of methylated, authentic GA_3 . In terms of ng per g dry weight of root tissue, the following quantities of GA_3 were recorded: upper halves: 130, lower halves: 63 (ratio upper: lower = 2.06); right halves: 70, left halves: 74. Qualitatively the distributions were thus similar to those found by the bioassays.

The direction of the concentration gradient of gibberellins in the horizontally positioned *Vicia faba* roots is opposite to that found in shoot tips and coleoptiles^{1,2}. The direction of the gradient is also opposite to that of auxin. Theoretically, gibberellin might thus assist in the positive geotropic reaction by stimulating the elongation of the upper half, provided that gibberellin does stimulate root elongation.

Pilet¹³, working with intact seedlings of *Lens culinaris*, found no effect on root elongation by GA_3 at concentrations up to $1 \mu\text{M}$, whereas 10 and $100 \mu\text{M}$ GA_3 retarded growth. There are, however, a number of reports indicating a stimulation by gibberellins of root elongation in some plants: *Pseudotsuga*¹⁶, *Lycopersicon* (excised)^{17,18}, *Triticum*¹⁹, and *Carthamus*¹⁹.

In our own experiments (to be reported in full elsewhere) GA_3 was added to germinating *Vicia faba* seeds when their radicles had just broken the seed coat. The root lengths were measured after 24, 36, and 48 h. GA_3 was found to be stimulatory at concentrations from 0.001 to 10 mg l^{-1} (except at 1.0 mg l^{-1} at 24 h). At all times 100 mg l^{-1} was inhibitory. Seedlings of *Lepidium sativum* were transferred to GA_3 solutions on filter paper when their roots were about 20 mm long. The root lengths were measured after 1, 2, 3, 4 and 5 h. In the first hour, 10 and 100 mg l^{-1} were inhibitory, whereas lower concentrations had no significant effect. At 2 h there was a stimulatory trend at concentrations from 0.1 to 10 mg l^{-1} , whereas 100 mg l^{-1} was inhibitory. At 3, 4 and 5 h, all concentrations from 0.001 to 100 mg l^{-1} were stimulatory, the lowest concentration being the most effective.

Gibberellin may thus have a function in root geotropism, but since the geotropic response in roots involves a retardation of the elongation of the lower part, the action of an inhibitor of root elongation in that part cannot be dismissed. Although such a role has traditionally been assigned to auxin, several authors⁴⁻⁸ leave open the identity of the actual functioning inhibitor. The presence in roots of an inhibitor which is not auxin, but becomes distributed like auxin in horizontal roots, has been demonstrated in this laboratory and its characteristics are being studied.

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Batesian mimicry without distastefulness?

MANY distasteful species are conspicuously coloured¹ and this is believed to aid their survival through the formation

of 'avoidance images'² by predators. It has been suggested that 'an efficient escape mechanism' either provided by a jumping response^{3,4}, or a fast escape flight⁵⁻⁷, could be as powerful as distastefulness in influencing a predator's strategy of prey selection, or could in some instances help to increase the relative frequency of an agile mimic of a distasteful but sluggish model⁸. Lindroth³ has made observations on several genera of flea-beetles (Alticinae, Chrysomelidae) and has found great external similarities between them and ground beetles of the genus *Lebia* (Carabidae), the larva of which is an ectoparasite of the flea-beetle's pupa. He suggests that the flea-beetle, which can escape suddenly from its exposed position on the food-plant by jumping, acts as a model and *Lebia* as a relatively sluggish mimic. As a parasite, *Lebia* is found in fewer numbers than the host, a situation also expected of some mimics in relation to their models. Neither was found to be distasteful to bird predators³. Thompson⁴ makes use of Lindroth's³ hypothesis in explaining the occurrence of an apparently aposematic form in females of the non-mimetic common meadow spittlebug, *Philaenus spumarius*, which is acceptable to predators but for the possession of an efficient close-quarter escape mechanism. Thompson proposes that this mechanism accounts for aposematic morph *P. spumarius marginella* occurring at a frequency in the population above that maintainable through apostatic selection. In both cases it is proposed that a learning predator comes to associate a particular colour pattern with wasted effort and spurns all forms with a similar appearance. This report describes a preliminary experiment to simu-

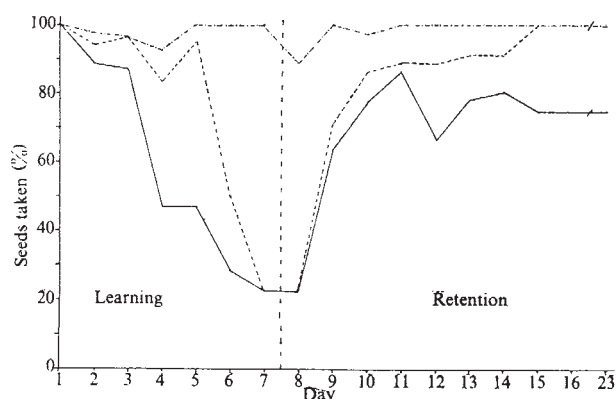


Fig. 1 Percentage of aposematic model/mimic, cryptic model/mimic and non-mimetic seed taken by the experimental birds during the learning period (model) and retention period (mimics). —, Green seed; ---, blue seed; —·—, red seed.

late this efficient escape and assess its effectiveness on a predator's selection of prey. *Bathilda ruficauda*, the star finch, was used as the predator and the prey was white millet seed dyed red, blue or green with food colouring (1% acetic acid base). The escape mechanism was simulated by means of a manually-operated hinged feeding platform which could be lowered rapidly to remove the seed from the birds' sight. The platform base was coloured with dots of blue and green dye, the same size as the seeds, and on this the red seeds appeared 'aposematic' and the blue and green seeds 'cryptic' to the human eye and presumably to the birds⁹. In front of the platform was a feeding perch and a light was turned on above to signal the presence of seed.

On each testing day the birds were deprived of food for 1½ to 2 h, transferred to the test cage individually (to prevent observation learning¹⁰) and presented with nine seeds, three of each colour, individually in random order.

The time taken to come to the feeding perch and make a peck at the seed was noted, an upper limit of 2 min being imposed for each seed. After this time the platform was dropped to remove the seed if it had not already been eaten. This operation was repeated three times a day for each bird.

Two groups of four birds were used, one control and one experimental group. The controls were allowed to feed on all seed colours for 3 d. In the experimental group the green represented the 'non-mimetic' on which feeding

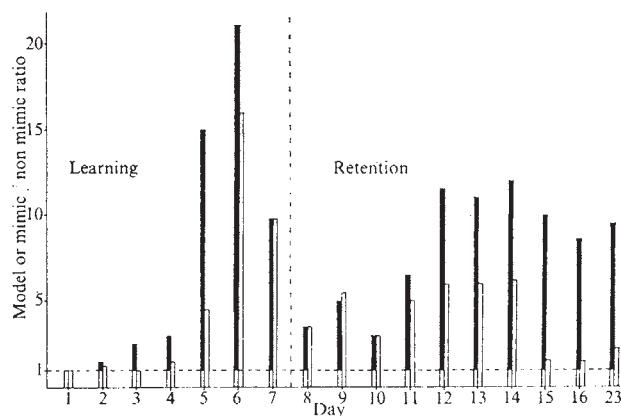


Fig. 2 The model-mimic/non-mimetic ratio (M/N Ratio) of mean feeding latency. A measure of the comparative advantage of the model-mimic complex over the non-mimetics. Feeding latency ratio; ■, red/green seed; □, blue/green seed.

was allowed throughout the experiment (days 1–16+23). The blue and red seeds acted as the 'models' during the first half of the experiment, the learning period (days 1–7), by being dropped out of sight as the bird attempted to feed. During the second half of the experiment, the retention period (days 8–16+23) the platform was not dropped for any seed until the end of the 2 min feeding time. The blue and red seeds were then being used as 'mimics'.

The results for the control group show that there was no colour preference in terms of mean feeding latency (Kruskal-Wallis one-way analysis of variance by ranks¹¹. $H=0.54$, $P=0.104$). The experimental group on day 1 also showed no colour preference (Fig. 1). During the learning period, discrimination took place between the non-mimetic (green) seed and the model (blue or red) seeds in terms of latency to feed. The aposematic (red seed) showed the greatest initial advantage over the non-mimetic (green seed), by being avoided before the cryptic model, and more often overall ($\chi^2=38.12$, d.f.=2, $P<0.001$), though the blue model also showed an advantage over the non-mimetic (green seed) ($\chi^2=26.4$, d.f.=2, $P<0.001$) (Fig. 1).

The use of daily mimetic advantage measurements (model or mimic/non-mimetic, feeding latency ratio) shows more clearly the relative advantage of the two model/mimics (Fig. 2); the aposematic model reaching the higher level on day 6. The figure also shows that the relative advantage of red over blue at first increases (up to day 5), then decreases to equality by day 7, the last day of the learning period; from day 11, the fourth day of the retention period, the aposematic mimic shows an advantage over the cryptic mimic. The existence of a mimetic advantage was also apparent when individuals were analysed separately (Table 1).

It was also noted that as the latency before taking a model/mimic seed increased so did the amount of activity unrelated to feeding, including flying around, moving along the perch and preening. A similar type of behaviour was

also exhibited by birds in an experiment on simulated distasteful Batesian mimicry¹² and is probably indicative of a conflict of behavioural tendencies in the animal. This slowing down of attack would give a real mimic more time to escape. Behaviour of this type was never shown towards the non-mimetic (green) seed by the experimental group, nor to any colour of seed by the control group.

The formation of an avoidance image² by the birds was due to the simulated escape mechanism and not to some feature of the apparatus, as the control group fed readily on all seeds from it and the experimental group went for the green seed without hesitation throughout the experiment. Avoidance image formation was therefore through 'frustration learning' which can be as effective as pain learning¹³, the mechanism operative in discriminating distasteful from palatable prey. It would seem then, that 'an efficient escape mechanism' as observed by Lindroth³ could be as powerful as distastefulness in qualifying its possessor as a model for Batesian mimicry. Further observations in the field may show the phenomenon to be less rare than is now supposed.

Table 1 Model-mimic/non-mimetic ratio for each bird during the learning period, retention period and both periods combined

Bird	Learning		Retention		Both periods combined (model/mimic complex)	
	B/G	R/G	B/G	R/G	B/G	R/G
B	2.7	4.1	4.0	7.8	3.5	6.0
M	6.8	10.7	4.0	4.5	5.5	7.8
WR	3.6	5.8	4.8	4.9	4.2	5.4
WL	2.5	3.5	2.1	2.8	2.4	3.3
All	3.1	4.7	3.8	5.7	3.3	5.0

B, Blue seed, (cryptic model/mimic); G, green seed, (non-mimetic); R, red seed, (aposematic model/mimic).

These findings also have implications for the phenomenon of balanced polymorphism in a prey population with aposematic morphs. Birds feed in a frequency dependent manner, the more common the prey the stronger the searching image¹⁴ formed. The selection of apostates (non-mimetic polymorphs) enables a prey species to occur in an area at a greater density than if it were monomorphic. Some of these morphs may appear aposematic and will create a searching image more readily than a cryptic morph. These aposematic forms, if in a species acceptable to a predator, will be found at a very low frequency in the population through apostatic selection mechanisms. *P. spumarius marginella* occurs in an area studied by Thompson⁴ at a level above that of other aposematic morphs, but below that of the cryptic morphs. As all four morphs have the escape mechanism the only difference is that *marginella* is strikingly coloured. In the experiment reported here, learning to avoid the aposematic model was more rapid and this learning was retained longer than for the cryptic model, though they had equally efficient escape mechanisms. In a natural situation the escape mechanisms would presumably be less than 100% effective but, as shown by the experiment, a predator would remember an encounter with the aposematic morph more readily than one with a cryptic morph³. If in a dimorphic species the aposematic form is, say, four times as easy to detect as the cryptic form then the strength of an avoidance or searching image formed would equal that formed by four of the cryptic form. In this simplified hypothetical situation with two morphs, a balanced polymorphic level would be reached when the cryptic morph was four times as common as the aposematic. If the aposematic morph becomes more frequent in the population 'the selective disadvantages of being conspicuous'¹⁴ and predation will increase until the aposematic morph is reduced to the balanced polymorphic level again.

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Temperature effects on hearing in two species of *Amphisbaenia*

SEVERAL recent studies have documented diverse auditory responses in lizards and turtles as a function of body temperature. Previous work was with iguanid lizards, which are diurnal, and can regulate body temperature by basking; and with the aquatic turtle *Pseudemys*. We have worked with amphisbaenians, which are highly modified burrowing reptiles.

Werner (refs 1 and 2, and personal communication) showed that the ears of six species of iguanid lizards were most sensitive in terms of the electrical potentials of the cochlea at temperatures corresponding either to the 'eccritic' or to the similar 'preferred' temperature. At this optimum temperature the species-specific auditory sensitivity curve is regular and has a characteristic zone of maximum sensitivity². Temperature shifts produce fairly predictable species-specific variations. A temperature drop impairs the sensitivity, especially for the high tones, and makes the function highly irregular, with many sharp variations in frequency. A moderate rise improves the response to high tones while reducing it for low tones. A further elevation is likely to have little effect on high-tone sensitivity, but that to low tones is seriously impaired. In the region of best sensitivity the change often approaches 40 dB. Thus a torpid lizard is likely to have poor perception of sound, even though this may signal a predator or other sort of danger.

A lesser temperature effect was found in the turtle, *Pseudemys scripta elegans*³. For a head temperature range of 14° to 33° C the response to a constant tone of 330 or 630 Hz varied up to 4.3 dB, showing two peaks of sensitivity, respectively at 19° and 29.5° C. According to Patterson *et al.*, temperature has two effects: it acts on the energy source for the generation of potentials and involves either a conductive mechanism or blood pressure.

An earlier study⁴ reported limited observations on a single specimen of the African amphisbaenid *Chirindia langi*. Cochlear potentials differed up to 18 dB in the region around 3,000 Hz between 24.4° and 29.4° C.

Observations on two other species of amphisbaenians now demonstrate strikingly different behaviour. The animals were

four *Blanus cinereus cinereus* (Vandelli) from the vicinity of Jerez de la Frontera, Spain, and three *Diplometopon zarudnyi* Nikolski: one from the vicinity of Damman, Saudi Arabia, and two from the vicinity of Kuwait. *Blanus cinereus* is probably the most primitive living amphisbaenian, and *Diplometopon zarudnyi* is a highly modified trogonophid.

Specimens were anaesthetised with ethyl carbamate (0.012 ml per g body weight, 20% in physiological saline), often supplemented. The cochlear potentials were recorded with a needle electrode in contact with the perilymph of the sacculle while stimulating with various tones over the range of 100 to 10,000 Hz. Temperature was monitored by a thermal probe deep in the cloaca and controlled within 1° C by means of an electric blanket draped over the animal. For details see ref. 4.

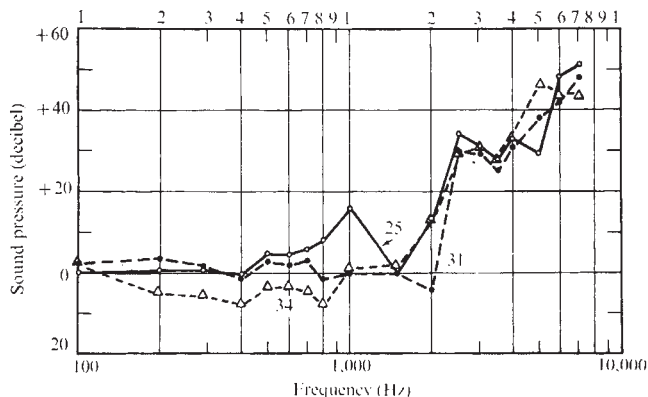


Fig. 1 Cochlear potentials of the left ear of *Blanus cinereus* (F868) taken at 25°, 31°, and 34° C in that sequence.

Figure 1 shows sensitivity curves (sound pressure in decibels relative to a pressure of 10^{-5} N m $^{-2}$, required at various frequencies to produce a standard potential of 0.1 μ V (that is, the lower the curve, the greater the sensitivity) for a specimen of *Blanus cinereus* at body temperatures of 25°, 31°, and 34° C. The curves differ, especially for the medium low and middle tones, but lack a clear trend as a function of temperature. Figure 2 shows similar observations on specimens of *Diplometopon zarudnyi* at four temperatures between 25° C and 40° C. All curves have the same general form.

A second specimen of *Diplometopon zarudnyi* tested at 37° C and then at 28° C yielded closely similar curves that cross one another several times. There was no obvious temperature effect, despite differences of 5–8 dB at two or three frequencies. The temperature was next set at 43° C, but the animal became hyperactive and a further test run had to be interrupted. After a period of time at a temperature of 35° C, a final series of measurements indicated reduced sensitivity. Possibly the high temperature of 43° C impaired the cochlear response.

These results reveal large differences among reptiles in the effects of temperature upon the response of the ear to sound. *Blanus* and *Diplometopon* maintain a remarkable constancy over a temperature range of as much as 15° C in sharp contrast to the lizards tested by Werner. *Pseudemys* and *Chirindia* evidently are intermediate.

It is as yet impossible to identify the process or processes involved in the observed temperature effects. Werner's observations seem definitely to exclude the sound conductive pathways, and point to the cochlear processes, and probably the hair cells themselves. The temperature effect may depend upon the magnitude of the polarisation potential set up at the surface membrane of the hair cell, and reflects the metabolic processes and membrane permeabilities by which this polarisation is established. Such possibilities should be tested and interspecies differences more broadly assayed. Do the responses reflect such environmental parameters as the range across

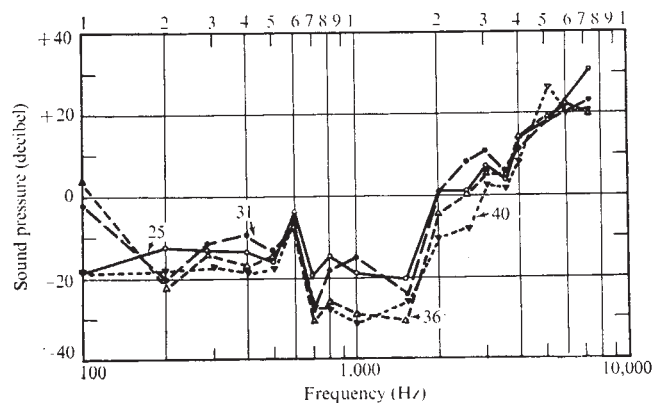


Fig. 2 Cochlear potentials of the left ear of *Diplometopon zarudnyi* (F869) taken at temperatures of 25°, 31°, 36°, and 40° C as indicated.

which a species normally thermoregulates? Can these amphisbaenians control the temperature of the otic capsule independent of general body temperature?

The sensitivity curves represented above show considerable variability tentatively attributed to the activity of middle ear muscles connecting to the terminal disk of the columella and probably affecting sound transmission to the inner ear⁵. Their activity may explain instability in the meter readings during presentation of a steady tone.

Efforts in one experiment to reduce this instability by the use of curare (*d*-tubocurarine) yielded uncertain results, perhaps because we were overcautious in the dosage used. Further experiments are needed on a more readily available amphisbaenian.

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Green cones of the piñon pine stimulate late summer breeding in the piñon jay

REPRODUCTION in birds is influenced by a variety of environmental factors, usually classified as ultimate or proximate¹. Ultimate factors determine efficiency of breeding, for example food supply for the young both before and after they become independent of their parents. Proximate factors, on the other hand, are the timers or predictors which initiate breeding at the appropriate time. Photoperiod is the most reliable and

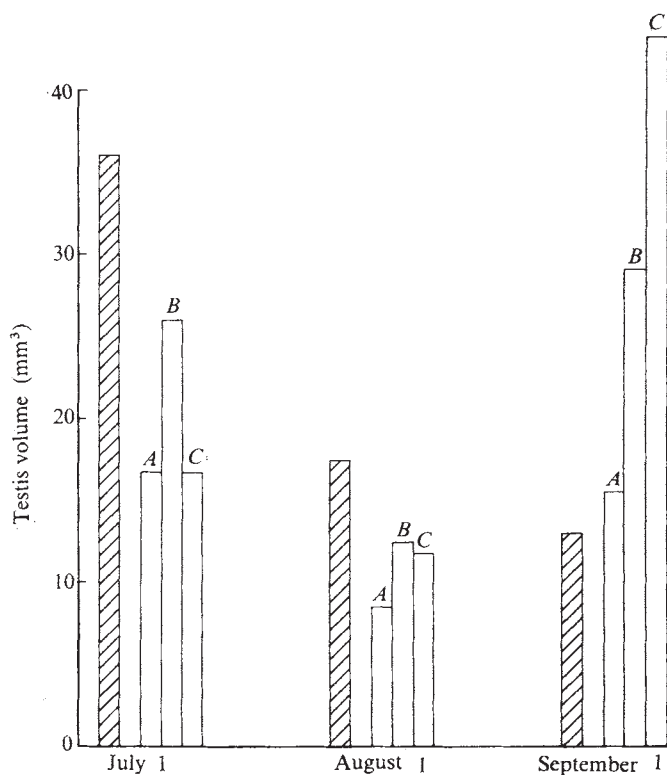


Fig. 1 Testicular response of three adult piñon jays provided freely with green cones of the piñon pine. Testes were regressing in ten experimental birds and ten controls through June and July. On August 1 the dried piñon seeds provided to the experimental jays were replaced with green unripened cones of the piñon pine. In three individuals shown here (A, B, C), testis regression was then reversed and the gonads enlarged. The unhatched bar represents maximum testis volume of the ten control piñon jays.

common proximate factor in those temperate zone species which breed exclusively in spring when daylength is increasing². Other proximate factors, such as rainfall, provide predictive information in many regions of the world, in that increased food availability follows the rains³.

Although most birds in temperate zones breed in spring and early summer, a few breed at other times of the year as well, in autumn or even winter⁴. In most such cases food abundance has been thought to be a primary stimulator of reproductive activity; however, with a single exception⁵, experimental support is lacking³. I report here field and experimental evidence that late summer-autumnal reproductive activity in piñon jays (*Gymnorhinus cyanocephalus*) of the south-western United States is triggered by the presence of large quantities of green cones of the piñon pine (*Pinus edulis*), which mature in late summer. Piñon pines in a given area produce cones and seeds largely in synchrony at irregular and infrequent intervals.

Piñon jays, like most other temperate zone bird species, are responsive to increasing photoperiod in spring (my unpublished work), and typically breed from late February or

early March to June⁶⁻⁸. In south-western New Mexico, however, near the south-eastern edge of this bird's range, climatic factors, and thus food availability, are unpredictable in spring and early summer and, as a result, breeding pattern and timing of the annual moult vary from year to year in a single population^{6,9}. It is here that late summer breeding sometimes occurs^{6,10}, and it seems to be related either to abundance of green cones of the piñon pine or to seeds contained within the cones (Table 1).

To test the roles of mature piñon seeds and the resinous green cones which contain maturing seeds, I spatially isolated two groups of ten adult male piñon jays in outdoor aviaries for 1 yr, beginning on September 1, 1971. One group received a nutritionally adequate diet, but with no piñon seeds or cones, whereas the other received the same diet, plus mature piñon seeds. The birds were laparotomised and their testes measured at monthly intervals.

No gonadal growth occurred in either group until the vernal increase in photoperiod, and through most of the year gonadal development was similar in both groups. By summer 1972 testes of all birds were regressing. On August 1, 1972, the ten jays receiving piñon seeds were instead given free access to intact, fresh, green piñon cones for a month. Regression of the testes was reversed in three birds (Fig. 1); that is, whereas dried piñon seeds did not prevent or delay regression of the testes, availability of green cones reversed gonadal regression and stimulated testis growth in some of the experimental birds. Gonads of the ten controls remained small (4.1–12.9 mm³, mean = 7.8 mm³), as did those of the seven experimental birds (1.4–12.5 mm³, mean = 7.8 mm³).

It is not surprising that most jays did not respond. The captive jays had neither mates nor suitable nesting sites, both of which are important in the attainment of reproductive competence in many birds¹¹. Further, in 1969, when autumnal breeding occurred, only about a third of the flock of about 300 jays actually bred⁶. As indirectly indicated in Table 1, natural selection favours no autumnal breeding in most years. Thus one could expect polymorphism in gonadal response to environmental stimuli in this population of piñon jays.

These data suggest that green piñon cones *per se* provide long term predictive information to the piñon jays; that is, the cones serve as proximate factors. In late summer, as a result of seasonal rains, other foods such as insects, are plentiful in every year, and it seems that piñon jays could easily provide for nestlings at this time. (It should be noted that piñon seeds form a very minor portion of the diet of nestlings^{6,12}.) Immature piñon jays become independent at about 8 weeks of age⁷. In birds hatched during autumn this occurs shortly before the onset of cold, intemperate weather. Great quantities of cones in August provide the proximate cue that adequate food, in the form of piñon seeds, will be available to the callow young during the stressful winter season.

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Table 1 Breeding phenology and testis condition of piñon jays in south-western New Mexico, in relation to production of seeds of the piñon pine

Year	Breeding phenology	Piñon cone production	Dates taken	Testis volumes (mm ³)*		
				N	Mean	Range
1969	No spring breeding, breeding in autumn	Abundant	19–20 Aug.	4	301.0	59.6–413.4
1970	Breeding in spring, none in autumn	None	25 Aug.	3	13.9	8.8–18.4
1971	No breeding, spring or autumn	None	5–6 Sept.	14	7.7	2.5–23.9
1972†	Some spring breeding, none in autumn	Light	2–3 Aug.	8	102.2	11.9–115.8
1973‡	Some late spring breeding, none in autumn	Light				

* Testis volume in cubic millimeters was calculated using the formula for the volume of an ellipsoid: $V = 4/3\pi a^2b$, where $a = 1/2$ the shorter diameter and $b = 1/2$ the longer diameter.

† Some gonadal development and associated behaviour took place in early August; however, nesting did not occur.

‡ Specimens were not taken but nesting did not occur.

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Pentamerism and the ancestral echinoderm

ONE of the difficulties in an account of the origin of the echinoderms is their five-fold radial symmetry. Nichols¹ has proposed a hypothetical ancestral echinoderm derived from a lophophorate ancestor. Sipunculoids have a recurved gut and a hydraulically operated tentacular system using a special compartment of the coelom. These resemblances to echinoderms suggest that a sipunculoid-like animal might have given rise to an ancestor of the echinoderms by developing a calcite skeleton for protection. This ancestor is illustrated in cross section in Fig. 1, but Nichols was unable to say why it should be pentamerous. On analysis of the requirements of such an animal, however, it has been possible to produce a 'paradigm' (in Rudwick's sense²) which demands five rays.

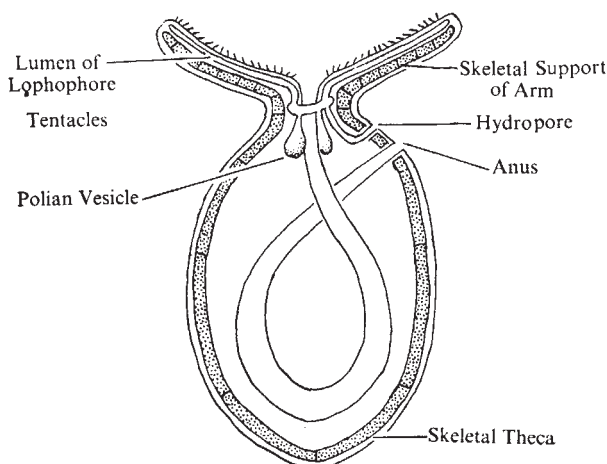


Fig. 1 Nichols' hypothetical ancestral echinoderm, in cross section.

Nichols' reconstruction, like many primitive echinoderms, has mouth, hydropore and anus in a straight line. Such an animal must live with the anus on the downstream side, to carry waste products away from mouth and hydropore (Fig. 2a). (In tidal waters, one current would probably be dominant, either because it would be stronger, or because it would carry food.) The hydropore fits best downstream of the mouth, where the current over it is filtered by the food-gathering apparatus. The lophophore of such an animal must serve three functions: first, to catch food settling vertically out of suspension; second, to catch food carried along horizontally by the current; and third, to detect the approach of danger.

The third function needs a ray extended directly upstream (Fig. 2a), which would also serve the first two functions to some extent, but more rays are needed for food gathering. These must be bilaterally symmetrical about the axis established in Fig. 2a, or the animal would be twisted by the current. An additional ray extending downstream would only be possible if it did not lie on the surface of the theca, as in an edrioasteroid, but was extended over hydropore and anus as an arm. This would be undesirable as the arm would hinder the current in carrying away waste products, and might create eddies carrying these products into hydropore or food groove. There must, therefore, be an odd number of rays; one extended upstream and pairs on either side of the axis: one pair of rays at right angles to the axis (Fig. 2b) catches food carried horizontally by the current, but makes no provision for catching food settling vertically or for detection of danger on the downstream side of the animal. These functions would be better served if all the rays met at equal angles (Fig. 2c). If each lateral ray is of length r , it would then sample a section of the current width $w = r \sin 60^\circ = 0.866r$. Each ray would also extend 'early warning' downstream from the centre by a length

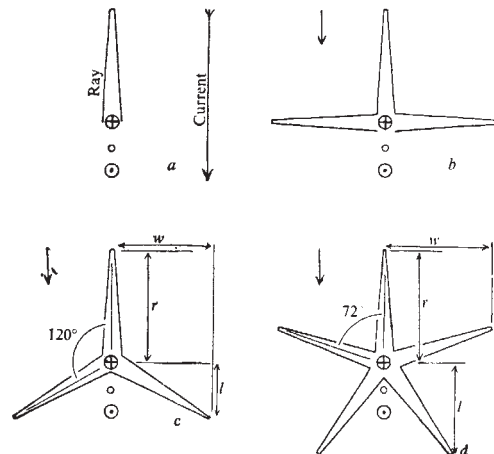


Fig. 2 Possible paradigms for an ancestral echinoderm. $\oplus$, Mouth; $\circ$, hydropore; $\ominus$, anus; r , length of ray; w , current width searched; l , extension of 'early warning' facility downstream. Short downward arrows in b, c, d represent current direction.

$l = r \cos 60^\circ = 0.500r$. But this is a vulnerable side, predators may be attracted upstream to the animal by secretions carried by the current.

If another pair of arms is added, the requirements of the paradigm can be filled more satisfactorily. The animal of Fig. 2d has five arms of equal length and at equal angles to their neighbours. It is symmetrical to the current with one ray upstream and the anus downstream; each of the anterior lateral rays samples a width of current of width $w = r \sin 72^\circ = 0.951r$ (and it can be shown that this advantage of 5 rays over 3 persists even if the current veers considerably); each of the posterior lateral rays extends warning backwards by a distance $l = r \cos 36^\circ = 0.86r$, while the anterior lateral rays guard the flanks; and finally, five equally spaced rays provide a better net to catch food settling vertically. The addition of a third pair of rays does not produce corresponding advantages.

Five rays like this might be evolved by the branching of the lateral rays of a triad³, or by repeated branching of the

single ambulacrum of an animal like *Helicoplacus*. However, Hyman's summary⁴ of sipunculoids as having "less than 10 to numerous" tentacles suggests that it might fit Nichols' theory better to postulate a reduction of rays to the acceptable minimum of five so that the animal expends the minimum of metabolic energy in calcifying them. This reduction would mean further loss of food-gathering power to a lophophore already restricted in flexibility by calcification, which could be overcome by the provision of lateral branches to the rays as outgrowths of the coelomic canal. Nichols deduced such branches, but from analogies with recent crinoids, he considered the branches would be respiratory. Selection might have favoured their development to meet the needs of both feeding and respiration. Whatever the exact details, the hypothetical ancestral echinoderm has now been equipped with pentameral symmetry and can give rise to a variety of primitive echinoderms.

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Fertilisation of sheep ova following their transfer to goats

AN interesting feature which has emerged from attempts to hybridise domestic sheep (*Ovis aries*) and goats (*Capra hircus*) is the marked difference in conception rate according to the direction in which the cross is made. In goats inseminated with sheep semen the conception rate is similar to that in goats mated naturally with male goats; whereas in sheep inseminated with goat semen fertilisation is an infrequent occurrence^{1–3}. The low fertilisation rate in sheep inseminated with goat semen has been confirmed by Hancock⁴ who found that only 5 of 93 (5.4%) sheep eggs were fertilised by goat spermatozoa.

Here I report an experiment undertaken with a view to isolating the factors which may be involved in the infertility of the sheep and goat cross, the approach being to circumvent any incompatibility between spermatozoa and environment by transferring sheep oocytes to the Fallopian tubes of goats which had been mated with fertile male goats. Results showed that most of the sheep eggs subsequently recovered had been fertilised and were undergoing cleavage, indicating that the sheep ovum presents no intrinsic barrier to the entry of the goat spermatozoon.

Eleven Border Leicester × Welsh Mountain ewes were treated with progestagen-impregnated intravaginal sponges (Veramix Plus; Upjohn Ltd) for 13 d, and were each given 1,500 i.u. pregnant mares' serum gonadotrophin (Folligon; Intervet Laboratories Ltd) by subcutaneous injection at the time of sponge removal. The ewes were then tested with a vasectomised ram twice daily for signs of oestrous activity, and were given either 6,000 µg luteinising hormone releasing factor (Roche Products Ltd) or 500 i.u. human chorionic gonadotrophin (Chorulon; Intervet Laboratories Ltd) by intramuscular injection,

18 h after the onset of oestrus. The animals were subjected to laparotomy approximately 24 h later. Six of the ewes were used as egg donors, the technique for recovery of eggs being that described by Hancock and Hovell⁵. The oocytes were transferred in a small volume of Hank's fluid into the ovarian end of the Fallopian tubes of four crossbred goats (G1, G2, G3 and G4) which had been mated with fertile male goats 30–60 min earlier. The remaining five ewes were inseminated with goat semen; the semen was collected with the use of an artificial vagina, and an equivalent of 2.15×10^9 (range 0.9–4.96) spermatozoa was deposited directly into each uterine horn. Goats G1, G3 and G4 and the inseminated sheep were subjected to laparotomy for egg recovery 3 d after the first operation; goat G2 was subjected to laparotomy 2 d after the first operation. A record was made of the number of corpora lutea in the ovaries of the goats at the time of the second operation.

Table 1 The numbers of sheep oocytes transferred to the left (L) and right (R) Fallopian tubes of mated goats, and the numbers of fertilised and unfertilised ova recovered

Goat	Sheep oocytes transferred		Corpora lutea		Ova recovered	
	L	R	L	R	L	R
G1	3	4	1	0	2 (6–8 cell) 1 (1 cell)	1 (5 cell)
G2	0	1	1	0	—	1 (2 cell)
G3	0	4	0	0	—	zona pellucida with spermatozoa
G4	3	2	0	1	2 (6–8 cell) 1 (1 cell)	1 (1 cell)

A total of six cleaved and three uncleaved ova were recovered from three of the goats (G1, G2 and G4). Assuming that, from the number of corpora lutea, one of the cleaved ova from goat G1 and one of the uncleaved ova from goat G4 were 'native' ova (see Table 1), the results indicate that seven of the transferred ova were recovered at the second operation. Five of the seven ova (71.4%) were cleaved. No ova were recovered from goat G4, but an empty zona pellucida showing the presence of embedded spermatozoa was found.

A total of ten ova were recovered from the five sheep in which goat semen had been placed directly into the uterine horns. None of the ova were fertilised, and there were no spermatozoa attached to, or embedded within, the zonae pellucidae.

Although the number of animals used in the experiment was very small, the fertilisation of transferred sheep oocytes in three goats and the presence of spermatozoa in the zona pellucida recovered from the fourth goat, indicate that there is no innate incompatibility between sheep ova and goat spermatozoa. These findings would seem to suggest that the low fertilisation rate in sheep inseminated with goat semen is due to factors affecting the survival, transport or capacitation of the goat spermatozoa in the ewe.

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Swamp cancer

HYPHOMYCOSIS DESTRUENS EQUI, also called swamp cancer or bursattee, is a granulomatous disease commonly of the lower leg of the horse and is a well known condition in the tropics and subtropics¹. The associated fungus, 'Hyphomyces destruens', has neither been legitimately described nor satisfactorily placed in any group of fungi except broadly in the Phycmycetes, on the grounds of the morphology of the hyphae in tissue and culture.

Following recent work in Australia by Johnston and Hutchins², a request was made to these authors for isolates of the fungus from the New South Wales cases, but none had survived more than a few months in culture. This fate has also occurred to the isolates of Bridges and Emmons (H. Hasenclever, personal communication). In September 1973 isolates were obtained from four cases of swamp cancer in horses in the Central District of Papua New Guinea, which closely agreed with the limited description available of 'H. destruens'.

Because of suggestions by Bridges and Emmons³ and by Amemiya and Nishiyama⁴ and its general resemblance to

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Erratum

In the letter "Appearance of unusual mitochondria in rice coleoptiles at conditions of secondary anoxia" by B. B. Vartapetian, I. N. Andreeva and A. L. Kursanov (*Nature*, **248**, 258; 1974) the wrong illustration was published as Fig. 1d. The bottom half of Fig. 1 should be:

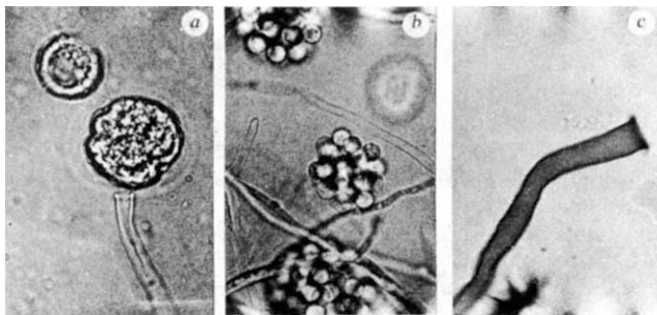


Fig. 1 a, Rotating vesicle of sporangial contents undergoing cleavage into zoospores immediately after emission ($\times 270$); b, zoospores encysted at sporangium apex ($\times 270$); c, sporangium aperture stained with Parker blue-black Quink ($\times 405$).

Mortierella spp., a number of methods developed to encourage these fungi to produce sporangia were tried out on the cultures, for example, hay and silage agars, but without success. A further attempt was made by placing portions of colonies from a Sabouraud glucose agar plate in sterile water in a Petri dish, to which a small piece of sterilised rotten maize silage had been added. After incubation for 2 d at 25° C biflagellate zoospores 9–10 μ m diameter were noticed arising from the cleavage of protoplasmic masses emitted from undifferentiated filamentous sporangia (Fig. 1).

This behaviour shows that 'H. destruens' is a Phycmycete belonging to the Pythiaceae in the Peronosporales and that it could be included in the genus *Pythium* Pringsheim⁵. Further work is in progress to establish whether it is a recognised or a new species, but the existence of a *Pythium* sp. pathogenic to mammals has not previously been suspected.

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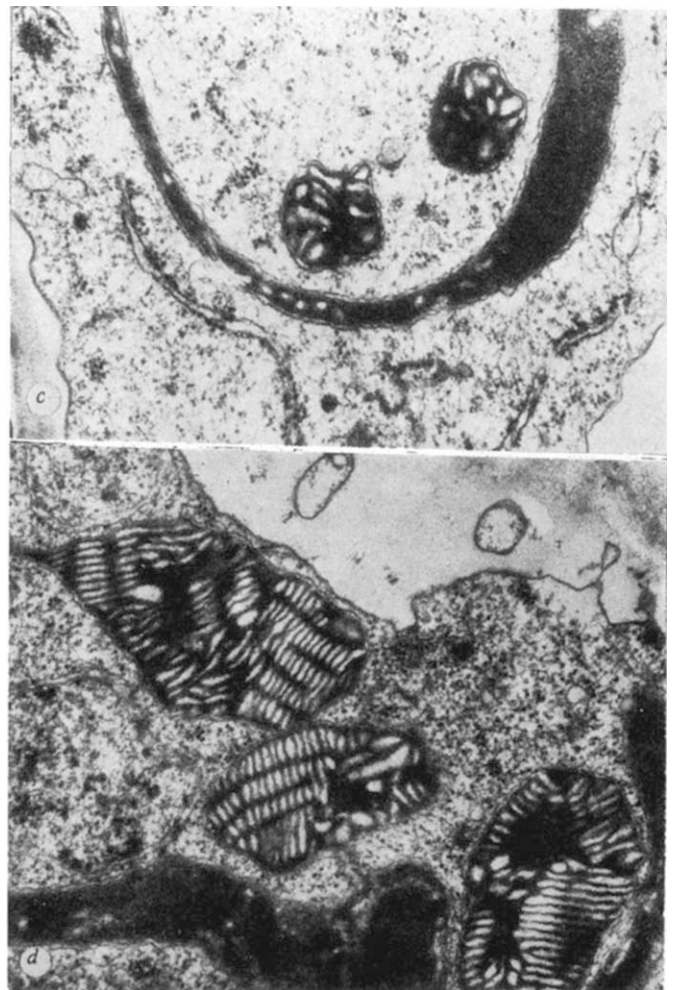


Fig. 1 Ultrastructure of mitochondria in cells of rice roots and coleoptiles in aerobic and anaerobic conditions. a, Root, 9 d after growth in aerobic conditions; b, root, 6 d of aerobic growth, then 3 d of growth in anaerobic conditions; c, coleoptile, 9 d of aerobic growth; d, coleoptile, 6 d of aerobic growth, then 5 d of growth in anaerobic conditions.

book reviews

Pastures new

Introduction to Ecology. By Paul Colinvaux. Pp ix+621. (Wiley: New York and London, 1973.) £5.80.

Textbook of Theoretical Botany. Vol. 4. By R. C. McLean and W. R. Ivimey-Cook. Pp. viii+3317-3912. (Longman: London, October 1973.) £12.00

Introduction to Plant Ecology: A Guide for Beginners in the Study of Plant Communities. By A. J. Willis. Pp. 237. (Allen and Unwin: London, November 1973.) £5.25 boards; £2.95 paper.

THE publication of E. P. Odum's *Fundamentals of Ecology* (Saunders, Philadelphia, 1959) heralded a new era in the teaching of ecology at university undergraduate level. In particular it was Odum's emphasis upon the ecosystem as the basic unit of ecology which permitted the novel but long-awaited integration of plant and animal studies within a single cover. At last due emphasis was given to the principles governing the flow of energy, the cycling of elements and the dynamics of populations of organisms within the physical constraints imposed by the climatic and edaphic environment. As a result, this book did more than any previous text to influence the development of ecological teaching in universities throughout the world. Traditional plant and animal ecologists have been inspired not only to unite in their research efforts but also in their attempts to convey to their students an understanding of the functional mechanisms at work in natural communities of plants and animals.

The positive feedback system thus created has inevitably resulted in a logarithmic increase in the information accretion rate within the newly unified discipline of ecology. As a result, principles require modification, techniques require revision and new and better examples of many fundamental concepts emerge, all of which serve to make any text less and less useful as a teaching tool with the passage of time. Consumer demand must eventually result in the revision or replacement of traditional textbooks. Odum's text has undergone periodic revision, most recently in 1971, but for several years there has been a need for a fresh look at ecology in its totality by a new author uncommitted to the highly evolved, but often rigid, framework of a previously

published text. Two such texts have recently migrated across the Atlantic to its more conservative right-hand seaboard. One of these, Paul Colinvaux's *Introduction to Ecology*, is to be reviewed here; the other, Robert Ricklefs's *Ecology* (Nelson, London, 1973) was reviewed in these columns last year (245, 342; 1973).

Colinvaux's book is thoroughly refreshing in approach, content and style. The arrangement of material is systematic and sensible. Beginning with plant and animal geography, the author places an unusual, but highly desirable emphasis upon the time factor in the development of the formation (=biome) both on the successional time scale and over longer periods involving climatic change. Colinvaux's informal, even anecdotal, style makes this section particularly easy and exciting to read—his selection of material is so skilled that the founders of biogeography emerge as human beings rather than the stuffy Victorian protagonists pictured in so many texts.

The second part of the book deals with the ecosystem as a unit and, inevitably, it leans heavily upon Odum. But once again the author's highly selective and analytical approach leads to simplicity, consistency and flow in his style. For example, in the opening section of part 2 he reduces the major contributions of Elton, Lindeman and Hutchinson to the development of ecosystem concept to a mere paragraph. The paragraph nevertheless remains readable, full of infectious enthusiasm, yet pregnant with information and meaning. This paragraph forms the basis for his further development of the themes of production, energy transfer and nutrient cycling.

Part 3 of the book deals with competition and the relationship of the individual to the population. Inevitably, this section contains an abundance of animal data, mainly because so few botanists have regarded plants in these terms. Colinvaux's approach is made uniform by the way in which he relates all of the work he reviews to the processes of natural selection and evolution. In the final part of the book he continues the evolutionary theme by asking some of those enigmatic questions which make modern ecology so fascinating; why are some species of organism common whilst others are rare, what controls diversity in ecosystems and how is diversity

related to stability of ecosystems?

It is easy to criticise this book on many aspects of content, particularly the lack of detail in some sections. For example, the descriptive analysis of plant communities (a subject which has riveted the attention of British and Commonwealth plant ecologists for decades, often to the exclusion of all other considerations) is treated very briefly when compared with that of, say, Kershaw's *Quantitative and Dynamic Plant Ecology* (Arnold, London, 1973). Genetic aspects of natural selection and evolution rate receive a less rigorous treatment than in Ricklefs's *Ecology*. But in my opinion, any such defects are greatly outweighed by the sheer readability of Colinvaux's text as well as the effective way in which it stimulates the most fascinating ecological questions in the reader's mind. Any teacher who needs a general ecological textbook to introduce this vast subject to undergraduates need look no further than this book.

McLean and Ivimey-Cook's fourth volume in their *Textbook of Theoretical Botany* is, as its name implies, a massive collection of information relevant to certain aspects of plant ecology and plant geography. One may be forgiven for questioning the relevance of such an encyclopaedic textbook in these days. No longer can the plant sciences be adequately covered in one text, even if it runs to five expensive volumes as this one is intended to do. Indeed, I would even question the need for a textbook embracing plant ecology at a time when ecological synthesis is proving so much more valuable than analysis in the construction of a conceptually based and predictive science of ecology. Even the short preface to this volume conveys to me an air of incipient defeatism: "Many people who meet this unfamiliar word (ecology) for the first time are moved to ask, 'what is ecology?' and 'what does it do?' The present sketch may be useful in answering such questions so far as plant life is concerned."

How, one may ask, can such questions be answered by reference only to plant life? They cannot, and this fact becomes increasingly clear as one proceeds through this book. For example, many conceptual terms are treated inadequately, simply because they depend largely upon animal studies for their existence. The term 'niche' cannot be explained solely with

reference to plants; the semi-quote from Odum that in is the plants' profession is meaningless. Colinvaux asked a much more searching question when dealing with this topic; why are there so many species of grass in a meadow when they are all doing the same job? McLean and Ivimey-Cook not only fail to answer such a question, they do not ask it. Again, how sad it is that seven pages of this concept section are devoted to 'physiognomy' and only one to 'ecosystem'. That single page contains many misconceptions and errors, for example, the confusion of the terms 'population' and 'community', and the idea that an ecosystem must be stable or it will "disintegrate and disappear". Primary production is mentioned here, though not defined, but one has to hunt within the 'edaphic environment' section in order to find two more pages devoted to this topic in the 800-page book.

The coverage of the volume is patchy. Production ecology is almost non-existent, but soils and related environmental factors are dealt with very extensively. Even here, however, there is a tendency to depend upon older literature for much material with resultant inaccuracy; for example, their treatment of organic soils and peats is antiquated and misleading.

It is a little surprising to find three habitats selected for more detailed treatment in a book of this kind. These are freshwater habitats, marine habitats and sea beaches all of which are covered by specialised texts. The use of the term 'sea beaches' is also misleading. Instead of a discussion of the maritime beach habitat one finds a brief account of intertidal benthic algae and their zonation. Here again terms are used loosely; 'emergence' and 'exposure' are both used where 'emersion' would be preferable. The effects of exposure (to wave action) are not dealt with at all, despite their profound influence upon intertidal ecology.

The second (and smaller) part of the volume is concerned with plant geography. This subject is dealt with in a far more satisfactory way than is plant ecology. The approach is generally historical and due emphasis is placed upon tectonic and glacial changes in Tertiary and Quaternary times in influencing the present pattern of plant distributions. There is a very useful section which discusses Adams's criteria for the recognition of centres of origin and distribution of plant genera. This would have been even more useful if it could have been married with some ecological principles. The separation of plant ecology from plant geography, like that of plant and animal ecology, is an historical accident the effects of which

have benefited neither discipline. Our efforts should surely be directed towards healing these breaches rather than preserving them.

One final criticism of McLean and Ivimey-Cook's book is the absence of a bibliography with each volume. Presumably one is expected to buy the fifth volume to benefit from the citations of specific publications in the fourth volume.

The third book under review is the revision by Willis of A. G. Tansley's *Introduction to Plant Ecology*. Having stated my basic reservations about books devoted to general plant ecology and to the revision and up-dating of classic textbooks, I need not labour them further. This revision is a thorough one and has involved the introduction of a large proportion of new material since the final 1954 edition of Tansley's book. At the same time the basic Tansleyan influence remains dominant in concepts and approach.

There are certain areas, however, in which it is impossible to do justice to modern concepts and still to retain the Tansley tradition. For example, the chapter on vegetational succession remains true to Tansley only by omitting the contributions of Deevey, Livingstone, Hutchinson and Odum. A large proportion of the book is given over to the description of vegetation, yet Tansley's ideas on this subject, although a milestone in the history of plant ecology, are now to be regarded in the way a modern radioastronomer would look upon Galileo's telescope. Similarly, a modern ecologist would not look to Tansley if seeking a means of describing vegetation. Can we not allow these classic texts to rest in respected peace upon our shelves and, inspired by their truths but liberated from the straight-jacket of their historical context, set about their replacement with yet greater works? I am sure that Willis and many other revisors would have been more gainfully employed in such a task.

A comparison of the three books under review here sadly reflects the development of ecology on the two sides of the Atlantic. The New World has set about the task of integrating the science of ecology while the Old World remains hide-bound by its much treasured tradition.

PETER D. MOORE

Generations of insects

Insect Population Ecology: an Analytical Approach. By G. C. Varley, G. R. Gradwell and M. P. Hassell. Pp. x+212. (Blackwell Scientific: Oxford and London, 1973.) £2.75.

THIS is an excellent book about insect population dynamics, with emphases on

the construction and evaluation of life tables, on quantitative description of the effects of parasitoids, predators and competitors, and on application of these techniques in the field of biological control. It will be valuable to students and research workers, especially to those working on insects with discrete generations, since this is a basic assumption of most of the models developed here.

It is not a complete text of population ecology, partly because many important ideas have emerged only in the study of vertebrates, and partly because the scope of the book is less wide than the title implies. Other facets of population ecology such as dispersal, insect-plant relationships, effects of environmental heterogeneity and evolutionary aspects are not to be found here. But these omissions are not shortcomings, since this comprehensible treatment of population dynamics is particularly valuable now when the subject is no longer controversial. The disputes of fifteen to twenty years ago were founded partly on difficulties in definition of terms, partly on misunderstandings of cause and effect, and partly on the tendency of researchers to generalise from their own results. Subsequently, in the early sixties, most writers were particularly careful to avoid these pitfalls, but this salutary effect seems to be wearing off. This book should help to reverse the trend once more.

Not all the deductions from population models are strictly accurate. A case in point concerns the authors' own work on the winter moth and its parasites. A high and variable proportion of winter moth larvae dies each year when eggs laid on oak twigs hatch before the buds burst, causing starvation of the larvae. P. P. Feeny whose work is not referred to here, has given evidence (*Ecology*, 51, 565-581; 1970) that the selection pressure responsible for this very early egg hatch stems from the accumulation of toxic chemicals in the oak leaves later in the season, such that larvae which hatched late would be unable to complete their development. If this is correct, then the interpretation given in the book—that the mortality is caused by "the wide scatter of bud opening times [which] prevents the time of hatch of winter moth eggs from being selected for very precisely"—is only half the story, and the assumption (page 133) that this mortality would be reduced if the buds opened synchronously on all the trees is incorrect. The same selection pressure for early hatching would still be present, and the resulting mortality would simply be more evenly distributed in space. The further assumption that this hypothetical situation would destabilise winter moth populations

seems equally unjustified, since, while the stabilising effect of parasites' responses to patchiness in host density would be removed, the major cause of instability—differences from year to year in the relative timing of egg hatch and bud burst—would, in the authors' interpretation, also be reduced.

M. C. SINGER

Obedient unto death

Obedience to Authority: An Experimental View. By Stanley Milgram. Pp. xvi+224. (Tavistock: London; May 1974.) £2.50.

"I WAS just obeying orders". From My Lai to Auschwitz and doubtless back to the time of Genghis Khan and before, the perpetrators of atrocities have excused their actions with some variation on that theme. Many people dismiss that excuse; they feel that the people involved in such horrors must be sadistic brutes not typical of the human race and comfort themselves with the secure knowledge that they would have the will and strength to refuse to obey such orders. Those comforting beliefs are shattered by Professor Milgram's account of his experiments at Yale during the 1960s. In the tests, subjects recruited from the general public to take part in what they believed to be a learning experiment were placed in a situation where they were required, under the orders of the "experimenter", to administer severe electric shocks to someone they thought to be another unsuspecting recruit for the learning experiment. Their willingness to do so is astonishing, and provides a new basis for understanding, if not excusing, the behaviour of people "just obeying orders".

Most of this book is taken up with descriptions of the various forms of the experiment and the responses of the subjects. Put baldly, it seems astonishing that anyone participated at all. Each volunteer was paired with an actor who was to be the victim in the experiment, and a rigged lottery ensured that the subject became the "teacher". The rationale of the test, as explained to him, was that the "learner" was to be encouraged to remember word pairs in a list by administration of electric shocks of increasing severity every time he made an error. The teacher had to administer these shocks while the genuine experimenter acted as the voice of authority. Many people were quite happy to administer what they genuinely believed to be shocks of up to 450 volts, while the learner screamed, protested, demanded to be released from the experiment and finally fainted.

Even when the teacher protested to the experimenter about the situation,

Fate, time, occasion, chance and change



Cartoon in *The Comic Muse*, July 18 1863, of the proposed removal of the Natural History collections to South Kensington (in fact not moved until the 1880s).

It hasn't always been 'all change' at the British Museum, but as readers of Edward Miller's scholarly and readable history (*That Noble Cabinet*, Deutsch, London, February 1974; £4.80) of the museum will soon discover, this famous institution has not been blessed with a particularly peaceful existence. For the first 150 years of the museum's life, since its foundation by Act of Parliament in 1753, if there weren't squabbles between the government, the trustees and the staff about money and buildings, there were worries about staffing, space and facilities; and in Victorian times especially there also seem to have been endless bickerings between certain of the senior staff.

The museum, however, weathered all the storms—including a heavy battering during the Second World War—and is all set to face the next

upheavals when, if present plans materialise, the library will be severed from the other departments and uprooted into new buildings. The museum was founded mainly as a department of natural history; ironically, it was this department—despised by the great librarian Panizzi (the architect of the present reading room)—which was the first major section to be severed physically if not completely emotionally from Bloomsbury when it was moved in the 1880s to Waterhouse's new buildings in South Kensington.

Miller—himself a museum man—has written a fascinating tale. There is no doubt that the BM has been favoured with more good luck, and loyalty from benefactors and staff, than its trustees and the British nation have at times deserved.

SARAH BUNNEY

he could usually be encouraged to carry on for some time, administering greater and greater shocks by such responses as "The experiment requires you to continue" or "It is absolutely essential that you continue". The voice of authority carried remarkable weight. So much so that the early simple versions of the experiment proved useless in defining exactly where people would draw the line. From having the 'victim' out of sight in the next room, successive modifications of the experiment eventually lead to physical contact, when the teacher had to hold the protesting learner in con-

tact with the plate supposedly giving shocks. And some people, acting under orders, carried out their duties up to the 450 volt level clearly labelled with "Danger Severe Shock". It is natural to ask how careful the experimenters were in their investigation—can their results be extrapolated to normal conditions? The answer seems to be yes. Over several years variations on the experiment were tried in which the "authority" was no longer respectable Yale University but a seedy (and fictitious) firm of "Research Associates", operating from a run down neighbourhood. Various permutations

on the roles were also investigated, with in some cases two experimenters giving conflicting orders, in others the experimenter himself offering to be the 'learner' to show how harmless everything was and then screaming to be let out, and so on. The results seem clear: given a defined chain of command and a guiding plan which must be obeyed most subjects were prepared to administer severe pain in a futile 'experiment' even when it caused them considerable emotional distress. In the most extreme cases, people were found who pressed on even when they thought the 'victim' had a weak heart and might be dying.

Thus far, the book is compelling reading and provides food not just for thought but for self analysis, but when Professor Milgram feels the need to produce theories to explain the behaviour he is on much weaker ground. This is as much because of the state of the art of psychology as through any fault of his, but it is a pity that able experimenters should so often feel the need to be theoreticians as well. The latter part of the book is, however, prevented from being in any sense an anticlimax by a double sting in the tail—an epilogue about My Lai, and an appendix discussing the ethics of carrying out the experiment at all.

MARY GRIBBIN

Eyes of the aged

The Human Lens in Relation to Cataract. Pp. x+324. (Ciba Foundation Symposium 19, New Series.) (Elsevier/Excerpta Medica/North-Holland, Associated Scientific Publishers; Amsterdam, London and New York, 1973.) Dfl. 43.50; \$16.70.

ALTHOUGH the surgery of cataracts figures prominently in many ophthalmic practices, clinical research has been mainly concerned with the technological problems of avoiding the post-operative complications. The relative success of modern cataract surgery may have inhibited to some extent the need for research into the mechanisms responsible for, and the changes which occur as a result of, the development of lens opacities in the aged. There cannot be complete complacency on the clinical side, however, as the patient after a successful lens extraction is nevertheless challenged by a series of problems in achieving 'better sight'. The need for research at a more fundamental level is thus of great importance.

Publication of this volume was timed to coincide with the retirement of Dr A. Pirie from the post of Reader to the University of Oxford, which she has held with distinction from 1948 to 1973, and records the proceedings of a symposium on the human lens

in relation to cataract under her chairmanship. In a series of papers followed by wide ranging discussions investigators eminent in their respective fields present their current views arising from investigations into the physical, biochemical, immunological and epidemiological changes which occur in the ageing lens. In subsequent sections it is perhaps unfortunate that the problem of iatrogenic cataracts did not receive more attention, but this was more than offset by a communication indicating that at least in certain parts of the world the age at which cataracts develop in populations depends on the geographical habitat.

This volume will be of value to cataract surgeons who wish to expand their knowledge of the subject beyond the confines of operative technique, and provides an up-to-date review of concepts and investigation techniques for laboratory workers engaged in research on lenticular opacities in the aged.

J. C. DEAN HART

Ions enter crystals

Channeling: Theory, Observation and Applications. Edited by D. V. Morgan. Pp. xii+905. (Wiley: London and New York, November 1973.) £11.75.

THE discovery in 1965 that under certain conditions fast ions from nuclear particle accelerators could penetrate to anomalously great depths in single crystals came as something of a surprise to physicists. It was not that any new principle was involved but simply that the passage of fast ions through solids had been well studied for some 70 years and it was not to be expected that a phenomenon as striking as channelling could have avoided detection for so long. This greatly increased penetration occurs when the charged particles travel along the directions of the strings of atoms which make up the crystal lattice, for in these directions the particles can travel freely in the open channels between the atoms with little energy loss. In the past eight years there has been a considerable amount of research on the channelling effect and the principal features are now well understood. Since the effect is beginning to find a number of applications the appearance of a comprehensive treatment is both welcome and timely.

The first half of the book is devoted to discussing the fundamental aspects of channelling and of the corresponding blocking phenomenon which gives rise to the distinctive pattern of ions backscattered by single crystals. Classical models provide a good account of these processes and computer simulation has been successfully employed for predicting the trajectories of ions

in crystals. With increasing accuracy of measurement, however, the use of a wave description may become necessary as in the case of channelling of electrons.

The second half of the book contains accounts of a number of the applications in which the phenomenon has been used. Thus since the blocking process depends sensitively on the structure of the solid it can be used for crystallographic analysis, the pattern immediately leading to the identification of the principal planes and axes of the crystal. Again impurities embedded in otherwise perfect crystals obstruct the free passage of the channelled ions and the nature and location of these impurities can be determined by the change in the pattern of the scattered ions. This technique of atom location is already finding many uses and includes the fields of radiation damage, surface physics and semiconductor technology, each of which is discussed at some length.

One historically interesting application lies in the field of nuclear physics and is for measuring time intervals as short as 10^{-18} s; this was devised in 1960 by Dr P. B. Treacy, five years before the phenomenon of channelling had been discovered. The principle of the method was to observe the distance travelled by an excited nucleus in a crystal in the interval between excitation and disintegration. Dr Treacy's assumption was that the decay products would have a greater chance of escaping from a crystal if the nucleus disintegrated at a point in a channel rather than at the lattice point where it was excited. His experiment proved abortive owing presumably to the poor quality of his crystals and no effect was seen. Some years later, however, when the channelling phenomenon was known, this application was independently proposed by other authors and shown to work. It has now become an established technique and some beautiful experiments have been carried out using it.

The whole field of channelling is excellently described in this book. Its multiple authorship ensures that the chapters on the different aspects of the field bear the stamp of authority and yet they are sufficiently linked together to make a coherent whole without destroying the pleasant idiosyncrasies in style of the individual authors; the editor is to be congratulated on his successful blending together of the component parts. It is informative, stimulating and well presented. As a library book it is likely to remain of value for some time and should be available to all. It will be of special interest to those working in the fields of solid state physics and of the science of materials.

M. A. GRACE

announcements

In the recently announced Honours List, **Sir John Wolfenden**, formerly chairman University Grants Committee and Director British Museum was made a Life Peer; **W. F. Anderson**, David Cargill Professor of Geriatric Medicine, University of Glasgow; **S. G. Clayton**, President, Royal College of Obstetricians and Gynaecologists; **A. F. Huxley**, Royal Society Research Professor, University College, London; **W. L. M. Perry**, Vice-Chancellor, The Open University; and **D. H. Wilkinson**, Professor of Experimental Physics, University of Oxford, were made Knights Bachelor.

Awards

The Society for Analytical Chemistry announces the **Robert Boyle Essay Awards**. (Entrants must be under 20.) For details write to: The Society for Analytical Chemistry, 9/10 Savile Row, London W1X 1AF.

Appointments

W. F. Vinen has been appointed to the Poynting Chair of Physics at the University of Birmingham.

D. R. Bates has been elected a Foreign Honorary Member of the American Academy of Arts and Sciences for his work on the study of the Earth's atmosphere.

William Lyall Ford has been appointed Professor of Experimental Pathology at the University of Manchester.

Ian Briercliffe Houston has been appointed to the additional Chair of Child Health at the University of Manchester.

Jean Kennedy McFarlane has been appointed Professor of Nursing at the University of Manchester.

J. Wiegold has been awarded a Personal Chair by the University of Wales.

Errata

In the article "Formation of methylmercury in a terrestrial environment" by W. F. Beckert *et al.* (*Nature*, **249**, 674; 1974) the units given in paragraph 3, lines 4 and 5 should be . . . 1 mCi ²⁰³Hg m⁻² and 2–20 Ci g⁻¹ respectively.

In the article, "Palatability dynamics of cardenolides in the monarch butterfly" by L. P. Brower (*Nature*, **249**, 280; 1974) Table 2 contained several errors and the following corrections should be made: California butterflies, line one, number of birds should read 9‡; line eight, absorbance range of subsample should read 0.320 to 0.399‖; Massachusetts butterflies, line four, number of birds should read 10¶¶; Totals should read Males 186 and Females 169.

International meetings

September 19, **Thermodynamics and Fluid Mechanics/Environmental Science and Technology Joint Conference** (A. J. Tugwell, Groups Department, The Institution of Mechanical Engineers, 1, Birdcage Walk, Westminster, London SW1).

September 19–20, **Action of Drugs on Vertebrate Cells in vitro** (Dr M. Balls, School of Biological Sciences, University of East Anglia, Norwich NOR 88C, UK).

September 20–22, **Science, Technology and Society in Victorian England** (The Centre for Continuing Education, Education Development Building, University of Sussex, Brighton BN1 9RG, UK).

September 20–22, **1974 Annual Conference of the Education Group of the Institute of Physics** (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

September 22–24, **Rosenhain Memorial Conference** (The Conference Secretary, Dr D. E. Miles, Division of Materials Applications, National Physical Laboratory, Teddington, Middlesex, UK).

September 22–25, **International Symposium on the Electro-optical Properties of Macromolecular Solutions** (Dr C. Houssier, Laboratoire de Chimie Physique, Université de Liège au Sart-Tilman, B-4000 Liège, Belgium).

September 22–25, **48th Annual Conference of Aslib** (The Conference Organiser, Aslib, 3 Belgrave Square, London SW1X 8PL).

September 22–28, **Short Course on Environmental Health Engineering in Hot Climates and Developing Countries** (John Pickford, University of Technology, Loughborough, Leicestershire, LE11 3TU, UK).

September 23–26, **2nd Meeting of the European Geophysical Society** (Professor A. Marussi, Istituto di Geodesia e Geofisica, Università Degli Studi, Trieste, Italy).

September 23–26, **International Symposium on Contamination Control** (ICCCS, 6 Conduit Street, London W1R 9TG).

September 23–27, **9th World Energy Conference** (E. Ruttle, Secretary-General, World Energy Conference, 5 Bury Street, St. James's, London SW1Y 6AB).

September 23–27, **6th International Symposium on Problems of Listeriosis** (Dr Malcolm Woodbine, Microbiological Unit, Department of Applied Biochemistry and Nutrition, Faculty of Agricultural Science, University of Nottingham, Sutton Bonington, Loughborough, LE12 5RD, UK).

September 23–27, **8th International Conference on the Properties of Water and Steam** (Conference I.A.P.S., Secrétariat, c/o S.F.T., 28 rue de la Source, 75016 Paris, France).

September 23–27, **6th International Congress of Infectious and Parasitic Diseases** (6th International Congress of Infectious and Parasitic Diseases, 01-201 Warszawa, Wolska 37, Poland).

September 23–27, **22nd Symposium of Vertebrate Palaeontology and Comparative Anatomy** (D. W. Yalden, Department of Zoology, The University, Manchester M13 9PL, UK).

September 23–October 3, **NATO Advanced Study Institutes Programme** (Miss C. H. Borrey, Institut de Recherche Interdisciplinaire, School of Medicine, Boulevard de Waterloo, 115, B-1000 Brussels, Belgium).

September 24, **Growth of Crystals from Biological Molecules** (Professor A. C. T. North, Astbury Department of Biophysics, University of Leeds, UK).

September 24–26, **5th International Symposium on Gallium Arsenides and Related Compounds** (Dr R. Veilex, Fifth International Symposium on Gallium Arsenide and Related Compounds, LEP, 3 avenue Descartes, F-94450 Limeil Brévannes, France).

September 24-26, **Radioimmunoassay and Related Topics in Clinical Biochemistry** (Mr J. Maxwell Jones, LKB Instruments Ltd., 232 Addington Road, Selsdon, South Croydon, Surrey CR2 8YD, UK).

September 24-27, **CAD 74 International Conference and Exhibition on Computers in Engineering and Building Design** (M. I. Dawes, IPC House, 32 High Street, Guildford, Surrey GU1 3EW, UK).

September 25, **Sounding Rockets and Experimental Results** (L. J. Carter ACIS, The British Interplanetary Society 12 Bessborough Gardens, London SW1V 2JJ).

September 25-29, **10th International Congress of Herpetology** (Dr K. Klemmer, Deutsche Gesellschaft für Herpetologie und Terrarienkunde, Senckenberganlage 25, Frankfurt (Main)-1, Germany).

September 26-27, **X-ray Astronomy by Rockets and Satellites** (L. J. Carter ACIS, The British Interplanetary Society, 12 Bessborough Gardens, London SW1V 2JJ).

September 27, **Annual General Meeting of the Bone and Tooth Society** (Royal Dental Hospital, 32 Leicester Square, London WC2).

September 28, **Nutrition Society Meeting** (Dr J. D. Sutton, Hon. Programmes Secretary, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, UK).

September 30-October 1, **Meeting of the Executive Committee of the International Union of Pure and Applied Physics** (Associate Secretary General of IUPAP, c/o Institute of Theoretical Physics, Fack, S-402 20 Göteborg 5, Sweden).

September 30-October 4, **2nd Symposium on Neutron Dosimetry in Biology and Medicine** (Symposium Secretariat, Gesellschaft für Strahlen- und Umweltforschung mbH, D-8042 Neuherberg/Munich, Ingolstädter Landstr. 1, Federal Republic of Germany).

September 30-October 5, **25th International Astronautical Congress** (International Astronautical Federation, 250 Rue Saint-Jaques, 75005 Paris, France).

Reports and Publications

Great Britain

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